EGG SHELL QUALITY AND MICROSTRUCTURE AS AFFECTED BY VITAMIN C, OTHER FEED ADDITIVES AND HIGH ENVIRONMENTAL TEMPERATURES

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(MET EEN SAMENVATTING IN HET NEDERLANDS)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE RECTOR MAGNIFICUS IR. F. HELLINGA,
HOOGLERAAR IN DE CULTUURTECHNIEK,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP VRIJDAG, 7 OKTOBER 1966, TE 16.00 UUR

DOOR

A. R. EL-BOUSHY

H. VEENMAN EN ZONEN N.V. – WAGENINGEN – 1966

THEOREMS

I

Ascorbic acid addition in the diet of the laying hen was effective to the maintenance and improvement of egg shell strength during periods of high and moderate temperature. So it is important to supplement the rations of layers in the warm months of the year.

II

Feeding amonium chloride to laying hens or exposing them to gas environment of high CO_2 caused thinner shells and a drop in blood A.R. It is advisable to supplement the feed with organic sodium compounds to raise the blood A.R. and improve shell quality.

III

Since the thickness of the shell membranes is highly correlated with breaking strength of the egg, further research is needed to determine the physical properties of those membranes.

IV

The mechanism by which vitamin C performs in improving egg shell quality appears to be mediated through the thyroid gland.

V

To improve poultry production in Egypt it is better to concentrate on the native breeds and improve them by different breeding systems, instead of importing foreign breeds.

VI

It is of great importance to carry on seaweed industry in Egypt (U.A.R.) for feed supplementation.

VII

In subtropical and tropical regions it is necessary to improve housing conditions in order to make broiler production possible during the whole year.

VIII

It is necessary to supplement the bread in Egypt with Zinc and Iron in order to avoid cases of anemia, hepatosplenomegaly, dwarfism and hypogonadisms in boys between the ages of 12 and 19 years old.

A. R. EL-BOUSHY
Wageningen, 7th October 1966

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CHAPTER 1

INTRODUCTION AND SCOPE OF THE STUDY

It is a well known fact that there is a continuously increasing demand for eggs of high quality. It has also been observed that the new hybrids of breeds, strains and inbred lines made possible a higher production of eggs which had to cover that demand. The main struggle between the producer, the dealer and the consumer concerns the delivery of an egg with a shell of high quality which will not crack during the different stages of marketing and retailing.

BUSTER (1948) reported that tremendous losses occur in the marketing of eggs. The total loss which occurs in eggs on their way from producers to consumers is estimated at approximately 26% of sales value, or 650000000 dollars in 1947 in the U.S.A.

It was also reported by RALSTON PURINA COMPANY (1957) that their losses incurred in the U.S.A. alone amount to some 100 million dollars per annum. Most of these losses represent reduced returns to producers, but some of it is born by wholesalers and consumers.

A large part of the losses can eventually be prevented, if the causes can be outlined and evaluated. One of the well known causes is the summer effect on the external shell quality. A lot of investigators came to the conclusion that summer is affecting shell quality. Among those investigators were WILHELM (1940) and ROMANOFF and ROMANOFF (1949).

To give an impression of the amount of losses which might occur, the following calculation can be made.

In the Netherlands for instance, the yearly total egg production in 1963 was 5340000000 eggs (Produktschap voor Pluimvee en Eieren, 1963). If the percentage of breakage had been as high as given by BUSTER for the U.S.A. (26%), the loss would have been. 1388400000 eggs in that year. From egg trading sources representing about 7.8% of the total production of the Netherlands, eggs received monthly and among those the number and percentage of cracked eggs are calculated and presented in table 1.

From these figures we can conclude that the total percentage of breakage on the way from the producer to the trader in only one year was about 4.4% of the total production. In the Netherlands this would amount to 235 millions of eggs with a value of about 28 million guilders (7.7 million $).

We can also conclude that the season is affecting the shell quality and the percentage of breakage, though in these circumstances the differences will be related to ageing of the flock rather than to high temperatures.

As a matter of fact, in the Netherlands the average monthly temperature in 1963 did not rise above 16.3 °C (July), so it is not likely that high temperatures in themselves had much influence on the percentage of breakage of eggs.

On the other hand it is well known that weak shells are a problem in hot countries like those in the Southern and Eastern parts of the Mediterranean sea.

TABLE 1. Shows the broken and cracked eggs received monthly in large egg trading enterprises.

Month	Eggs received	Number of broken or cracked eggs	%
January	34631030	1225261	3.5
February	30969030	1155879	3.7
March	37990003	1427614	3.7
April	28323960	1198250	4.2
May	28666226	1234724	4.3
June	35871440	1517317	4.2
July	28950000	1353498	4.7
August	29840000	1303264	4.4
September	41240000	1447571	3.5
October	36710000	1196653	3.2
November	38162000	1174437	3.0
December	44222000	1396939	3.1
Total	415575589	18158378	4.4

In those circumstances considerable losses may occur because of the adverse influence of high temperatures on shell quality.

Because of these losses caused by hot weather it was worthwhile to investigate the effect of cold and hot climate on shell quality of the hen's egg, and the possibilities to improve shell quality under those unfavourable conditions in order to produce a rather suitable resistant shell which will be profitable for both the producer and the consumer.

CHAPTER 2

INFLUENCE OF TEMPERATURE ON SOME EGG CHARACTERS

2.1. Introduction

Many factors are known to affect egg shell quality; they are heredity, nutrition, (e.g. calcium, phosphorus, manganese and vitamin D), the laying cycle, age of the bird and climate.

The adverse influence of high temperatures on shell quality is suggested to be related to a reduction of the metabolic rate and of the ability to synthesise egg shell constituents in conditions of heat stress. The reduction of total blood calcium under heat stress may in itself cause a reduction in shell deposition.

Since vitamin C has been shown to be involved in the utilization and metabolism of both organic and inorganic constituents in bone it seemed probable that its role in egg shell formation could be traced back to either the utilization or the metabolism of these constituents or to both of them. This study was carried on to investigate the effect of high and low temperatures and vitamin C supplementation on egg and shell characters and total blood plasma calcium.

2.2. Review of literature

2.2.1. *Influence of temperature on some egg characters*

2.2.1.1. Egg weight

Bennion and Warren (1933) reported that:

1. With fluctuating temperatures the mean weekly egg weight when compared with the mean maximum weekly temperature showed a sharp decline when the temperature was above 85 degrees F.
2. The mean daily egg size of birds placed under controlled temperature was reduced from 15 to 20 percent by application of high temperatures. The egg size declined much more rapidly under high temperatures than it increased when the temperature was lowered.

Romanoff and Romanoff (1949) stated that egg weight records collected in various latitudes, extending from the equatorial to Scotland, indicate that extremely low temperatures have little effect on the size of the egg. However there appears to be a consistent decrease in egg weight whenever the maximum temperature exceeds 21 °C for a few consecutive days. In cold climates, where the temperature seldom remains above this level for any length of time, there is only a little decline in the size of eggs laid in summer. In tropical or semitropical regions such as the Philippine Islands, temperature varies within a rather narrow range throughout the year, and egg weight is also fairly constant. Also Skoglund et al (1951), and Hutchinson (1953) showed a sharp decline in egg weight when the temperature was above 85 °F.

On the other hand ROSENBERG and TANAKA (1951) reported that in Hawaii where the maximum daily air temperature ranges around 80°F throughout the year, egg weight is not reduced, suggesting that moderately high temperatures alone do not adversely affect egg weight.

BENNION and WARREN (1933), BRUCKNER (1935), WARREN and SCHNEPEL (1940), HUSTON et al (1957) gave as an explanation for the decrease of egg weight in high environmental temperature, that laying hens exposed to high environmental temperatures consumed less feed than similar groups held at moderate temperatures.

Finally MUELLER (1961) reported from his study on the effect of constant and controlled cycling temperatures on egg weight, that a constant temperature of 90°F depressed egg weight as compared with a constant temperature of 55°F. He also added that egg weight in the cycling environment from 55°F to 90°F and back to 55°F was significantly lower than in a constant 55°F environment, but significantly better than in the constant 90°F environment. Concerning vitamin C additions and egg weight, THORNTON (1960) reported that there is no definite conclusion regarding increasing egg weight by vitamin C additions.

2.2.1.2. Specific gravity of the egg

There is no doubt that eggs laid by the same bird posses a somewhat similar specific gravity. OLSSON (1934) stated that the percentage of shell in a hen's egg could be accurately determined by the specific gravity test, and that this method could be used to determine shell thickness without injury to the egg. He found the specific gravity of the whole egg to range from 1.085 to 1.090 immediately after it was laid and that of the shell to be 2.325 ± 0.0149.

The specific gravity of the egg shell is nearly twice that of the egg contents. The entire egg's specific gravity is therefore largely influenced by the proportional amount, or thickness of the shell (OLSSON, 1934) as shown below by data on turkey eggs after PHILLIPS and WILLIAMS (1944).

Average specific gravity	Range in thickness of shell (Millimeter)
1.070	0.28–0.30
1.080	0.33–0.36
1.090	0.38–0.41

Specific gravity is highly correlated with shell thickness and also with breaking strength. BAKER et al (1958) and GODFREY (1949) both reported a highly significant positive correlation between the last two items.

As far as seasonal changes are concerned, environmental temperatures are manifested by a variation in the egg's specific gravity from 1.087 in winter to 1.078 in summer in the temperate zone (ROMANOFF and ROMANOFF, 1949). A similar trend has been observed in the Phillipine Islands (FRONDA, CLEMENTE and BASIO, 1935), where the egg's specific gravity ranges from about 1.072 in January to 1.031 in March. However, some workers have recognized the possi-

bility that low values found in summer in the temperate zone or during the dry season in the tropics may be due to loss of weight caused by evaporation of the egg content before the determination of specific gravity.

2.2.1.3. Breaking strength

The relative fragility of the chicken egg is important because of the financial loss that may be sustained by excessive breakage during handling and transportation. To a great extent, the breaking strength of the shell is influenced by the same factors that are responsible for variation in shell thickness.

MORGAN (1932) calculated a positive correlation between the shell weight and the breaking strength. NIVIKOFF et al (1949) and GODFREY (1949) reported a highly positive correlation between specific gravity and breaking strength. Also the correlation between breaking strength and shell thickness was found by many workers. GODFREY (1949) and RAUCH (1959) reported a high positive correlation coefficient between breaking strength and shell thickness of + 0.919 and + 0.55 respectively for the two workers.

As far as the environmental effect on breaking strength is concerned, HEUZER and NORRIS (1946) reported that breaking strength is greatly influenced by environment, it is lower during July and August than during the winter.

2.2.1.4. Deformation

SCHOORL and BOERSMA (1962) reported a method for measuring the shell strength without breaking the shell by the deformation method. They reported highly significant negative correlations between deformation and shell thickness in the different points of the egg surface. Also a high negative correlation was found between breaking strength, shell percentage and deformation under pressures far lower than breaking strength.

The estimations of correlation coefficients from some shell characteristics after SCHOORL and BOERSMA (1962) were as follows:

$rux = +0.625$ $(P < 0.01)$
$ruy = -0.882$ $(P < 0.01)$
$ruz = +0.629$ $(P < 0.01)$
$rxy = -0.758$ $(P < 0.01)$
$rxz = +0.849$ $(P < 0.01)$
$ryz = -0.803$ $(P < 0.01)$

u = Breaking strength
x = Shell thickness
y = Deformation under 500 g pressure
z = Percentage shell

2.2.1.5. Shell thickness

It is generally known that egg shells are of greatest and most uniform thickness in winter and become thinner during the spring and summer. BENNION and WARREN (1933) recorded the fact that the egg shells seemed to be more fragile when the birds were subjected to high air temperature. WARREN and SCHNEPEL (1940) obtained thinner egg shells almost immediately after experimentally increasing the environmental temperature from 20 °C to 32.5 °C. A recovery in shell thickness occurred after a subsequent decrease in temperature. They also

observed that high humidity accentuated the effects of high temperature, and that the feed consumption of the hens was 27 per cent less at 32.5°C than at 20°C.

Also WILHELM (1940) and BRANT et al (1953) reported from their work on high environmental temperature and shell thickness, that the usual pattern followed is a gradual thinning of the shell, which is initiated during the spring months and becomes progressively worse as the temperature increases; when the atmosphere cools there is a return towards normal thickness.

CONRAD (1939) gave a reasonable explanation for the thinning of the shell thickness of birds subjected to high temperatures. He mentioned that shell secretion is probably retarded at high temperatures by the diminished intake of calcium in the feed, and possibly also by a reduced capacity of the blood stream to carry calcium.

2.2.1.6. Shell weight and percentage of shell

The total weight of the egg is not always distributed in the same way among the three chief component parts, yolk, albumen, and shell. By weight, the egg of the hen consists roughly of six parts of albumen, three parts of yolk and one part of shell.

Of all parts of the egg the yolk shows the least seasonal fluctuation in actual weight (PHILFOTT, 1933). The albumen shows the greatest seasonal variation in proportional amount (CURTIS, 1914a), while the weight of the shell declines, both absolutely and proportionally during the warmer months of the year. The following data indicate the relation between decrease in shell weight and increase in average maximum temperature (HEYWANG, 1946).

Temperature	Shell weight	
	Actual	Proportional
in °C	in grams	in percent
19	5.6	8.8
23	5.4	8.5
32	4.9	8.0
39	4.2	7.1

An artificial increase in the temperature of the environment of the hen demonstrates the effect of undue heat upon the proportional composition of the egg. The egg and all its component parts decrease in actual weight. The yolk gains relatively, but the albumen and shell suffer a proportional loss, the shell to a great extent (BENNION and WARREN, 1933a). Of all the components of the egg, the shell shows the greatest variability in amount.

WILHELM (1940) reported that temperature appears to influence dry weight of the shell and percentage of shell of total egg weight.

MUELLER (1961) reported from his experiments at constant temperature of 55°F and 90°F that the shell percentage was significantly decreased in the temperature of 90°F.

2.2.1.7. Shape index

ROMANOFF and ROMANOFF (1949) reviewed the literature on the shape of the egg and causation of shape. They reported that the shape of the egg can be approximately indicated by the ratio between breadth and length. The shape index is relatively independant of absolute size and varying contours; a relatively long and narrow egg of any size will have a low index, and a short and broad egg, whether large or small, will have a high index.

STURKIE (1965) defined the shape of the egg as the width-length index of the egg. A higher index means a shorter, more oval shaped egg, and a lower index, a longer narrower egg. It is believed that pressure exerted on the egg in the oviduct determines its shape. Opinions differ, however, as to what part of the oviduct influences shape most. Egg shape is influenced most by the isthmus, according to some, and by the uterus, according to others.

As much as environment and seasonal variation is concerned, BENJAMIN (1920) noted that the eggs of White Leghorns, both pullets and hens, were roundest during the natural breeding season. The index value increased from 69 in December (the first month of production at that time) to 72 in March, and subsequently declined to 70.5 in September, when laying ceased. While it was reported by Stichting voor het Fokkerijwezen bij de Pluimveehouderij te Zeist (1962–1963), that with proceeding laying season, shape index declines with increasing egg size. On the other hand, MARBLE (1943) was unable to demonstrate significant seasonal fluctuations in the shape of the eggs laid by individual Barred Plymoth Rock hens, or by the flock as a whole. His data showed only a slight trend towards rounder eggs at the same time of the year during which BENJAMIN noted the highest index.

2.2.1.8. Formation period of the egg

Formation period is influenced by different regions of the oviduct. The following table (after WARREN and SCOTT, 1935b) indicates the average time spent in each region of the oviduct.

Region of oviduct	Duration of stay		Proportion of total time per cent
	hours	minutes	
Infundibulium	–	20	1.4
Albumen-secreting region	3	00	12.8
Isthmus	1	10	5.0
Uterus	19	00	80.0
Vagina	presumably very brief		

From these figures it is clear that the shell formation in the uterus takes nearly 19 hours. The length of the laying interval, therefore, is determined chiefly by the number of hours the egg spends in the uterus (WARREN and SCOTT, 1935b).

WARREN en SCOTT (1935a, 1935b) reported that eggs laid within 25 hours of

the previously laid egg remained in the uterus 18.0 hours where as an egg produced in 30 hours remained in the uterus 21.6 hours.

BERG (1945) reported that increases or decreases in the time interval between eggs within a clutch, is accompanied by corresponding increases or decreases in shell thickness. He also reported that with the 3, 4, 5 and 7 egg clutches the correlation between the clutches and shell thickness was highly significant, and with the 6 egg clutches it was significant, indicating that differences in the thickness of egg shells of a bird may be due to differences in the time elapsed in formation of the eggs.

VAN ALBADA (1958) observed a significant increase in shell thickness under a 26 hours light which increased the minimum interval between eggs within a clutch from 24 to 26 hours approximately.

2.2.1.9. Clutch size

Several hypotheses have been advanced to explain the size of the clutch. It has been stated (FOX, 1899) that the number of eggs laid in a clutch bears a definite relationship to the amount of danger to which the species is exposed and that tropical species lay fewer eggs than those in the temperate regions.

TAYLOR and LERNER (1939) showed that the second egg of a clutch has a thinner shell than the first egg and that it is possible to eliminate the differences in thickness of shell of the two eggs by selection and breeding. WILHELM (1940) concluded that there is little decrease in shell thickness between the first and last eggs of the same clutch of 1, 2, 3 and 4 egg clutches. Although he did not show any conclusions from the fact, WILHELM's data indicated that the first and last eggs of the three and four egg clutches are thicker than the shells of the intervening eggs.

Shell thickness and position in the clutch have been observed by BERG (1945) who reported from his observations a considerable variation in the thickness of the shells produced from one day to the next. It was further noted that the shells of the first and last egg of cluthces of three or more eggs were usually thicker than the intervening eggs. He also added that the shell of the second egg of the two egg clutches studied was thicker than that of the first and last eggs of longer clutches. In clutches of three or more eggs, the shells of the first and last eggs were thicker than those of the intervening eggs.

2.2.2. *Blood calcium and external egg quality*

The shell of an average egg contains 1.5–2.0 g calcium, most of which is deposited during the 15 hour period immediately prior to oviposition (BURMESTER, 1940; WARREN and CONRAD, 1942; BRADFIELD, 1951), and calcium is, therefore, withdrawn from the blood at the rate of at least 100 mg/hour during the main period of shell calcification. A 2 kg hen has a plasma volume of approx. 100 ml (STURKIE and NEWMAN, 1951), and taking 25 mg/100 ml as an average figure for the level of total calcium in the plasma of a laying bird, it appears that during the period of active calcification an amount of calcium equal to the total quantity circulating at any instant is withdrawn every 15 min. Changes in the blood

calcium level associated with shell formation have been investigated by KNOWLES, HART and HALPIN (1935), and more recently, by POLIN and STURKIE (1957), and WINGET and SMITH (1958). The results of these different studies are very conflicting and it is still not possible to state with any degree of certainty what changes occur in the blood calcium during the laying cycle. Finally, HERTELENDY and TAYLOR (1961) reported that on the normal Ca diet, a highly significant fall in total plasma calcium was observed during shell calcification.

Concerning the effect of high temperature and humidity on blood calcium and egg shell, BENNION and WARREN (1933) and WARREN and SCHNEPEL (1940), reported that shell thickness is reduced markedly if hens are subjected to high environmental temperatures. Their experiments further suggested that the depressing effect of high temperature is accentuated by high humidity. The mechanism or mechanisms which cause poor shell quality at high air temperatures are still not definitely established. CONRAD (1939) found that oxalated whole blood calcium levels were reduced at high environmental temperatures. The following figures show these relations:

Temperature °F	60	87	87	75
Relative humidity %	55	80	40	65
Whole blood calcium mg/100 cc	19.12	14.28	12.48	17.56

He also reported that in general, an increase in temperature from 70 °F caused a relative decrease of 25–30 percent in the blood calcium level, and he added that the effect of high temperatures on the thickness of egg shells can probably be attributed directly to the decreased calcium 'carrying capacity' of the blood. From this evidence WARREN and SCHNEPEL (1940) concluded that 'the decrease in shell thickness when the birds are subjected to high temperatures is probably due to a reduction in calcium content of the blood'. They have also reported that hens kept at 95 °F consumed about 26 % less feed than hens kept at 60 °F. It is possible that this reduction of feed intake is at least partially responsible for the poor shell quality at high air temperatures.

On the other hand, MUELLER (1959) reported from his work on serum calcium, shell thickness and environmental temperature, the following figures.

Temperature °F	85	55	85
Relative humidity %	70	70	25
Shell thickness (mm)	0.373	0.400	0.384
Average serum blood calcium content mg % (laying pullets)	21.2	23.4	22.8

He also reported that serum calcium levels in the variable environment were significantly higher than those in the constant environments. In the constant environments, pullets kept at 85 °F and 70 % R.H. had significantly lower serum calcium levels than pullets kept at 85 °F and 25 % R.H. or 55 °F and 70 % R.H. It was concluded that the reduction in shell thickness at high air temperature

and humidity was not due to the coincident lowering of feed intake and serum calcium levels.

As far as the plasma calcium is concerned, WINGET et al (1958) reported that the analysis of plasma from the arterial and venous blood of the shell gland indicates that circulating calcium is removed by this organ during shell formation. This organ has an efficiency of 20% in removing circulating calcium both in the early and late stages of calcification. The concentration of diffusible calcium in the plasma does not appear to be affected by passage through the shell gland.

2.2.3. *Vitamin C supplementation and shell quality improvement*

Besides the well established records on the multiple physiological activities of ascorbic acid in humans and guinea pigs, it was also found in poultry that supplementation of hen's diets with ascorbic acid during the hot season resulted in a lot of improving results concerning egg size, egg production and shell quality.

THORNTON and MORENG (1958) reported that shell thickness was increased by supplementation of ascorbic acid in amounts of 5 mg, 10 mg and 20 mg per pound of ration. He added that this effect was more pronounced during the time that environmental temperatures were high (82°F). THORNTON and MORENG (1959) came to the conclusion that ascorbic acid is effective in partially preventing normal decline in feed consumption and egg shell thickness in the chicken during periods of heat stress. He added that ascorbic acid may have had an influence on the thyroid activity, of a stimulatory nature, particularly under the conditions of increased environmental temperature.

SULLIVAN and GEHLE (1962) reported that ascorbic acid slightly decreases calcium levels in the blood. Therefore, ascorbic acid may increase egg shell thickness by promoting the transfer of serum calcium to the shell gland of the oviduct. SULLIVAN and KINGAN (1962) came to the same results, significantly.

On the other hand many workers came to contradictory results in this aspect. HEYWANG and KEMMERER (1955) reported that under hot conditions no appreciable change in egg weight or shell thickness occurred when the diet was supplemented with ascorbic acid. THORNTON (1960) reported that the influence on egg weight of vitamin C addition was inconsistent at the different calcium levels; therefore, no definite conclusions can be made. ARSCOTT et al (1962) found no improvement in egg shell thickness as measured by specific gravity in Leghorn Layers fed rations containing 10 mg/lb ascorbic acid under natural environmental conditions, but they found a marked improvement in shell thickness accompanied by an increase in blood calcium and phosphorus for normal size birds in the presence of a diet containing 3% calcium compared with a diet containing 2.25%. Also HUNT and AITKEN (1962) reported an increase in egg numbers but no improvement in egg shell quality by using dietary ascorbic acid (20 mg/lb). Also PEREK and KENDLER (1962) reported that egg shell quality was not significantly improved by using a basal diet plus 25,75 and 400 ppm of vitamin C respectively in a temperature of up to 110°F with a fluctuating relative humidity between 18 and 95%.

2.3. Material and methods

This work was carried out in the different Institutes and Departments belonging to or co-operating with the Agricultural University of Wageningen in The Netherlands.

The first experiment was carried out in the Department of Animal Physiology of the Agricultural University and in The Central Institute for Poultry Research 'Het Spelderholt', Beekbergen, Department of Product Research.

The second experiment was carried out in the Department of Animal Physiology, mentioned before.

The third experiment was carried out in the Institute of Agricultural Research of Biochemical Products (I.L.O.B.). The last part of the work was done in the Soil Survey Institute, Micropedological Department.

The first experiment started in December 1963 and ended in June 1964. The main object of this study was to notice the effect of cold and hot environment and of vitamin C suplementation in the food on the shell quality of the hen's egg in combination with a microscopical study of the structure of the eggshell.

Experimental animals: 48 single comb white Leghorn chickens (linebred) of about 7 months old were used in this study. A small number of reserve animals were kept for completion of experimental groups in case of the death of birds.

Housing: In this experiment the environmental facilities used consisted of two darkened rooms, one cold and the other hot. Both rooms were artificially illuminated each with two incandescent bulbs of 100 watts attached to a time switch providing illumination 14 hours per day. The cold room was equipped with an air conditioning unit which was circulating and cooling the air of the room to a temperature of 55 °F with a range of ± 5 °F and keeping the relative humidity on 50–60 %. Ventilation was provided by an exhausting fan. The hot room was equipped with four electric heaters 2 kW each fitted with a blower. The heaters were thermostatically controlled. The temperature in the room was kept at 85 °F with a range of ± 5 °F. The source of humidity was an electric boiler which was working constantly and supplying the room with a basic humidity of about 50 % R.H. Additional humidity was provided by a hygrostatically controlled electric sprayer bringing the R.H. to 75–80 %. In this room too there was an exhausting fan for ventilation. Temperature and humidity were registered continuously by a thermohygrograph.

Two experimental rooms were situated in the basement of the laboratory which had naturally a rather constant climate. In each room there were 24 hens in a battery equipped with automatic waterers.

Feeding: Feed was offered in troughs, birds were fed *ad libitum*. Since calcium percentage in a layers ration is well known to have an effect on shell quality, it was taken into consideration to raise the percentage of calcium in the ration from 2.25 %, (originally the normal allowance [1]) to 2.75 % in order to avoid

[1] The official National Research Council (1962) allowances has since been raised to 2.75 % (Nutrition Requirements of Poultry Number I, 1962).

any possibility of having a weak egg shell during the experiment from calcium deficiency.

The feed formula and composition is given in table 2.

TABLE 2. Composition of all mash laying ration.

Ingredients	Amount (kg)
Ground yellow corn	37
Barley meal	20
Oatmeal	17.5
Wheat bran	2
Soybean oil meal (solvent extracted)	5
Corn gluten feed meal	3
Fish meal (68% protein)	4
Alfalfa meal (18% protein)	4
Vitamin B mixture[1]	1
Vitamin A.D_3 (750 IU D_3 2250 IU A/g)	0.2
Mineral mixture[2]	2
Precipitated chalk	4.4
Di calcium phosphate $2H_2O$ (calcium content 23%)	0.5
Manganese sulphate	0.007
Zinc sulphate	0.020
	100.627

[1] *Contents of Vitamin B mixture*

Riboflavine	200	mg/kg
Panthocenic acid	400	„
Niacine	1500	„
Choline	40000	„
Vitamin B_{12}	0.5	„
Vitamin E	200	„
Vitamin D_3	100	„

[2] *Contents of mineral mixture %*

Calcium carbonate	48.85
Calcium phosphate	37.85*
Sodium chloride	12.00
Zinc sulphate	0.3
Copper sulphate ($CuSO_4.5H_2O$)	0.2
Manganese sulphate	0.8

* Ca 25%. $Ca_3(PO_4)_2 + CaHPO_4.2H_2O$

Calculated chemical composition of the ration.
Crude protein 15.1%. Productive energy kcal/kg 1850. Metabolizable energy kcal/kg 2650. Crude fibre 5.8%.
Calcium 2.75%. Phosphorus 0.68%. Manganese 75 mg/kg. Zinc 70 mg/kg.
Methionine 0.24%. Cystine 0.22%. Lysine 0.65%. Arginine 0.77%.

Experimental design

A survey of the experimental design is given in table 3.

Control period: At the beginning of the experiment during one month in both rooms the same natural climatic conditions were kept to check the equality of the groups and to have an opportunity for making statistical corrections for group differences.

Acclimatization period: Because of retardation in the delivery of the heating equipment after the control period, a period was inserted in which no artificial regulation of the climate was applied, except that provided by central heating of the building and additional gas heating and an electric boiler for vapour supply in the hot room. During this period the hot room was heated during the day from 8 a.m. to 6 p.m. only at 85°F and R.H. 75–80%.

Stress period: After the acclimatization period there was a period with nor-

TABLE 3. Experimental design.

Date	Treatments	No. of birds	Cold room temperature and humidity %	No. of birds	Hot room temperature and humidity %
December 1–31 1963	Control period	24	65–70 °F 50–60 R.H.	24	65–70 °F 50–60 R.H.
January 1 to February 29 1964	Acclimatization period	24	55 °F 50–60 R.H.	24	day 10 hr 85 °F 75–80 R.H. night 14 hr 65–70 °F 50–60 R.H. (Fluctuating temperature)
March 1–15	Transition,				65–70 °F 50–60 R.H.
March 15–31 1964	no data available	24	55 °F 50–60 R.H.	24	85 °F 75–80 R.H. (Fluctuating temperature)
April 1–30 1964	Stress period	24	55 °F 50–60 R.H.	24	85 °F 75–80 R.H.
May 1 to June 30 1964	Vitamin C additions and stress	No. V.C. 12 / V.C. added 12	55 °F 50–60 R.H.	No. V.C. 12 / V.C. added 12	85 °F 75–80 R.H.

mal heating of 15 days for some technical reasons. After that the stress period started for another 45 days of which the first 15 days till the beginning of April was without collection of data. In this period the temperature in the hot room was kept continuously at 85°F. Collection of data was performed under the same conditions from April 1 to 30.

Vitamin C additions: In the last part of the experiment from May 1st till June 30th, the groups of birds in each room were split at random in two lots of which one was treated with vitamin C addition to the feed, whereas the other served as a control. Each group was composed of 12 layers. The vitamin C which was used was in the form of L-ascorbic acid ($C_6H_8O_6$) one of the HOFFMANN-LA ROCHE products. It was added to the ration in a concentration of 50 mg per kilogram after premixing. The same ration used in table 1 was prepared weekly, stored in a cold basement and offered to the birds twice a day in order to avoid losses by oxydation as much as possible.

To be on the safe side, stability tests were carried out to determine the effect of storing on vitamin C concentration. The determination of vitamin C was carried out in the Department of Rural Home Economics Section of Food and Nutrition. Vitamin C was photometrically determined (methods of vitamin assay 1951). The following determinations show the effect of storing the mixed feed with 100 mg/kg for one day, one week and two weeks at 65–70°F and 60% R.H.

One day storing	11.58 mg/100 g
	11.42 mg/100 g
One week storing	13.48 mg/100 g
	14.85 mg/100 g
Two weeks storing	11.96 mg/100 g
	12.26 mg/100 g

These data show that loss of vitamin C during the experiment can be ruled out.

Sampling procedures: All eggs were collected hourly every day in the whole experiment in order to calculate the formation period. After each hourly collec-

tion the eggs were placed in a humid cold container in order to avoid losses by evaporation.

Methods of determination:

I. Specific gravity:

At 4 p.m. eggs were weighed on a Mettler balance type H_3 to the nearest one hundredth of a gram. After weighing, specific gravity was determined by weighing the egg under water at 20 °C. For this the same balance was used with a special attachment. During weighing in water air bubbles were avoided to ensure the correct reading. Specific gravity was calculated as follows:
Egg weight – Egg weight in water = Egg volume
specific gravity = Egg weight/egg volume.[1]

II. Other determinations of shell quality:

Eggs were stored for shell quality determination for two weeks and then they were sent to Beekbergen[2] for the following determinations:

1. shape index
2. deformation
3. breaking strength
4. shell thickness
5. shell weight
6. shell percentage.

1. Shape index: shape index is normally determined by the following equation.
   Shape index = Breadth/Length × 100.

Generally it is measured by an outside caliper for measuring the breadth and the length. In this study ,shape index was determined by a semi-automatic apparatus (VAN DOORN) which gives a direct reading of the index, without measuring breadth and length separately.

2. Deformation: This is a rather new method of determination of the resistance of the shell against deformation without damaging the shell. It was measured with a special apparatus designed by SCHOORL and BOERSMA (1962). This apparatus measures the bending of the egg, under a certain load (500 g). The deformation was measured on the equator part of the egg. Measurement on that place was chosen because the shell at the equator shows less variability and may thus give a higher correlation coefficient with the shell thickness, than when the measurements are taken on the large or pointed end of the egg. Readings of the deformation are in 0.001 mm.
3. Breaking strength: The measurements of breaking strength were made with a device constructed especially for this purpose at Beekbergen. This apparatus is designed in a way that a pressure is applied on the egg by a bolt placed on the large end of the egg. The source of pressure was by means of granulated lead shot. When the lead was poured, the pressure on the shell was produced. As

[1] For sake of simplicity no temperature corrections were made, so all specific gravity data are related to water temperature of 20 °C.

[2] Central Institute for Poultry Research 'Het Spelderholt'.

soon as the shell is broken the lead stops pouring automatically; the quantity of lead poured is weighed on the same apparatus giving the breaking strength in kilograms and grams.

4. Shell weight and shell percentage: Eggs were broken, the inner contents were removed; the shells were rinsed carefully with luke warm water to clean them from the traces of albumin, then they were dried at 105 °C for 3 hours and weighed twice till a constant weight on the Mettler balance to the nearest 0.01 mg.

Shell percentage was calculated as follows:

Shell percentage = shell weight/egg weight × 100.

5. Shell thickness: Shell thickness was determined in three parts of the shell; the blunt end, the equator and the pointed end (shell membranes inclucded). The measuring of the thickness was done with an outside micrometer with a pointed anvil and a rounded spindle (VAN DOORN) to the nearest 0.01 mm. Readings were taken by averaging three measurements on the circumference of the different parts.

III. Plasma blood calcium determination:

Blood samples were withdrawn from the wing vein with a very sharp needle and a syringe. 2 ml of blood were enough for the determination of calcium in the plasma. A special table was used to attach the bird in an easy way to take the sample, heparin was used as an anticoagulant. Blood samples were withdrawn between 1 p.m. and 3 p.m. only from hens which had laid an egg on the day of the sampling. Calcium was determined by complexonmetric method using the Vitatron apparatus. Samples of blood were taken monthly over the whole experiment from December 1963 till June 1964.

2.4. RESULTS AND DISCUSSION

Egg weight:

Table 4 and 4a show the means and the F values of the analysis of variance and covariance in the different periods of the experiment. Figure 1 shows the course of the mean egg weight in the different periods of the experiment.

It is clear from these data that there was an increase in egg weight in the cold climate in the periods from 1 till 7 due to the ageing of the birds.

The group treated with vitamin C showed a lower rise in egg weight from the beginning till the end of the trial. No significant effect of vitamin C was noticed.

Concerning the fluctuating temperature or acclimatization effect, no significant differences were noticed though a slight depressing effect of higher temperatures on egg weight can not be ruled out. The stress effect of the 85 °F temperature however resulted in a clear drop of egg weight which was highly significant. Vitamin C addition diminished the decline in egg weight in hot climate but this effect was statistically not significant. Concerning the stress effect at the 85 °F temperatures, many workers, such as BENNION and WARREN (1933), ROMANOFF and ROMANOFF (1949) came to the conclusion that high temperature decreased egg weight significantly. An explanation was given for the decrease of egg weight

TABLE 4. Egg weight as affected by environmental temperature and vitamin C additions.

Months	Experimental periods	Mean egg weight (g)				L.S.D. between[1] single groups	
		Cold environment		Hot environment			
		Control	Treatment	Control	Treatment	$P < 0.05$	$P < 0.01$
1	Control	55.39	53.95	55.27	55.48	1.49	1.96
2	Acclimatization	58.04	56.04	56.92	57.73	1.49	1.96
3	Acclimatization	59.98	57.82	57.45	58.33	1.53	2.02
4	Transition			No data available			
5	Stress	61.68	59.45	54.45	56.46	1.78	2.33
6	Vitamin C add.	62.82	59.89	52.67	56.31	2.00	2.63
7	Vitamin C add.	63.47	60.18	53.47	55.52	1.89	2.48

TABLE 4a. Analysis of variance and covariance in the different periods separated and combined showing the effect of treatment, temperature and their interactions on egg weight.

Months	Experimental periods	Calculated F. Value[2]			
		Kind of analysis	Treatments	Temperature	Interactions
1	Control	A.V[3]	0.66	0.85	1.18
2	Acclimatization	A.V	0.61	0.14	3.43(*)
3	Acclimatization	A.V	0.67	1.67	3.74(*)
4	Transition	–	–	–	–
5	Stress	A.V	0.02	31.79**	5.47*
		A.Co[4]	–	56.02**	–
6	Vitamin C	A.V	0.12	45.09**	10.33**
	additions	A.Co	1.55	81.56**	7.44**
7	Vitamin C	A.V	0.41	57.82**	7.66**
	additions	A.Co	0.01	97.85**	4.55*
6 & 7	Vitamin C add.	A.Co	0.36	100.01**	7.09*

[1] L.S.D. = Least significant difference.
[2] Degrees of freedom = Treatments, temperature and interactions 1, error 44.
F 10%(*) = 2.84 F 5%* = 4.06 F 1%** = 7.27.
[3] A.V. = Analysis of variance.
[4] A.Co. = Analysis of covariance against 1&2&3 combined.

in high environmental temperatures that heat stressed birds are consuming less feed than similar groups held at moderate temperatures. However it was shown by BENNION and WARREN, that the reduction of feed consumption by 50% did not effect egg weight. It is more likely that the stress on water balance by excessive evaporation is responsible for the reduction in egg weight under heat stress.

Concerning the fluctuating temperature, MUELLER (1961) reported that an environment with temperatures cycling from 55°F to 90°F was significantly

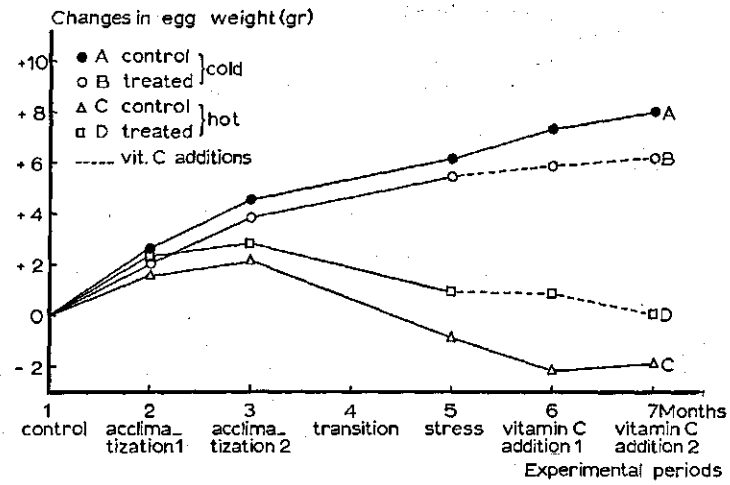


FIG. 1. Changes in egg weight from the initial values during the course of environmental temperature changes and vitamin C additions.

better than a constant temperature of 90°F. These findings are confirmed by the results obtained in the acclimatization period.

As far as the effect of vitamin C on egg weight in hot environment is concerned, THORNTON (1960) reported that there is no definite conclusion regarding increasing egg weight by vitamin C additions.

The significant interactions seen here may be due to the unequal distribution between the four groups, since these interactions were present already to a certain extent, before the vitamin C treatment started. A larger number of birds would be needed to prove whether an effect of vitamin C in the hot climate is present or not.

Deformation:

From table 5, 5a and figure 2 we can see that in the cold climate a steady increase in deformation occurs when the birds became older. As far as hot temperature is concerned, fluctuating (acclimatization) temperature has no significant effect on deformation. On the other hand the heat stress effect was highly significant. Vitamin C supplementation seemed to improve the mean shell quality in the hot environment as measured by deformation. This difference was nearly significant, but since the group receiving vitamin C at the end of the experiment showed a lower increase in deformation also in the stress period (without vitamin C), no clear proof is obtained that vitamin C realy improved shell quality as measured by deformation. Irregularities, which may be due to the rather small number of birds (12 per sub group) are indicated by the nearly significant interactions already present in period 5. In the cold environment no effect of vitamin C can be seen.

Deformation is inversely associated with shell quality. Correlations between deformation and other shell quality characters are shown in table 6. On the hen

TABLE 5. Egg shell deformation as affected by different environmental temperature and vitamin C additions.

Months	Experimental periods	Deformation μ (with 500 g pressure)				L.S.D. between[1] single groups	
		Cold environment		Hot environment			
		Control	Treatment	Control	Treatment	$P < 0.05$	$P < 0.01$
1	Control	21.2	21.4	21.3	21.3	1.39	1.82
2	Acclimatization	22.8	23.1	22.7	22.2	1.60	2.11
3	Acclimatization	22.7	24.4	24.1	22.6	2.00	2.63
4	Transition			No data available			
5	Stress	28.1	29.2	37.0	32.9	1.71	3.07
6	Vitamin C add.	30.4	30.8	39.6	33.6	3.61	4.75
7	Vitamin C add.	32.3	33.0	39.7	33.8	3.31	4.37

TABLE 5a. Analysis of variance and covariance in the different periods separated and combined showing the effect of treatments, temperature and their interactions on egg shell deformation.

Months	Experimental periods	Calculated F. Value[2]			
		Kind of analysis	Treatments	Temperature	Interactions
1	Control	A.V[3]	0.01	0.00	0.02
2	Acclimatization	A.V	0.01	0.41	0.24
3	Acclimatization	A.V	0.01	0.05	2.30
4	Transition	–	–	–	–
5	Stress	A.V	1.00	16.15**	2.86(*)
		A.Co[4]	–	25.93**	–
6	Vitamin C additions	A.V	2.31	10.66**	3.10(*)
		A.Co	2.91(*)	13.90**	2.45
7	Vitamin C additions	A.V	2.42	5.90*	3.71(*)
		A.Co	3.78(*)	9.99**	3.27(*)
6 & 7	Vitamin C add.	A.Co	3.99(*)	14.35**	3.07(*)

Foot notes: See table 4 a.

average basis deformation is highly correlated with breaking strengh, shell thickness, specific gravity of the egg and shell percentage. This is in agreement with the findings of SCHOORL and BOERSMA (1962). The highest correlations are shown with shell percentage and shell thickness on the equator, where deformation was measured, indicating that on a hen basis, deformation is a reliable estimate for shell percentage primarily. On an individual egg basis (calculated for periods 6 and 7 only), the correlations are much lower, though still highly significant in most cases. This indicates that the correlations on a hen average basis may be partly dependent on genetical factors.

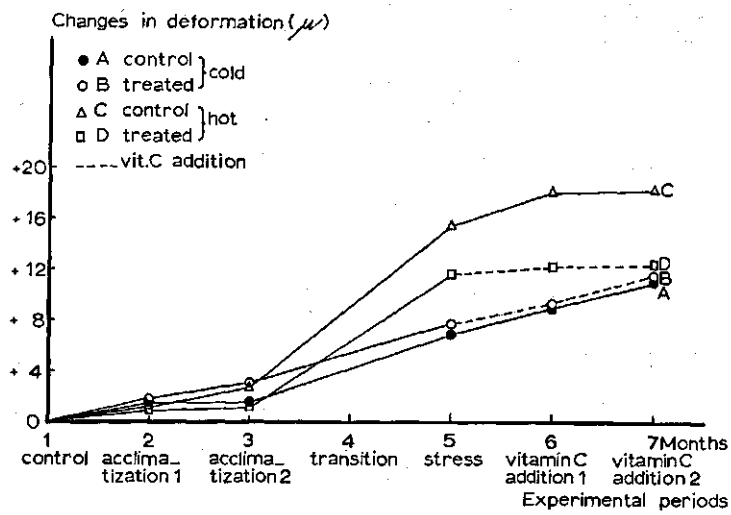


FIG. 2. Changes in deformation from the initial values during the course of environmental temperature changes and vitamin C additions.

TABLE 6. Correlation of deformation with different shell characters based on hen average corrected for temperature and treatment effect.

Months	Experimental periods	Breaking strength	Specific gravity	Shell thickness				Shell percentage
				Blunt	Pointed	Equator	Mean	
1	Control	–0.643**	–0.428**	–0.792**	–0.745**	–0.886**	–0.875**	–0.697**
2	Acclimatization	–0.602**	–0.786**	–0.652**	–0.694**	–0.835**	–0.739**	–0.856**
3	Acclimatization	–0.685**	–0.860**	–0.731**	–0.741**	–0.904**	–0.836**	–0.912**
4	Transition	–	–	–	–	–	–	–
5	Stress	–0.774**	–0.864**	–0.793**	–0.771**	–0.851**	–0.848**	–0.899**
6	Vitamin C add.	–0.669**	–0.915**	–0.675**	–0.815**	–0.846**	–0.825**	–0.926**
7	Vitamin C add.	–0.670**	–0.819**	–0.682**	–0.698**	–0.785**	–0.712**	–0.894**
	Correlation of deformation with different shell characteristics based on egg averages for months 6 and 7 corrected for temperature and treatment effect.							
6 & 7	Vitamin C add.	–0.367**	–0.594**	–0.456**	–0.394**	–0.554**	–	–0.362*

* Significant at 5% level. ** Significant at 1% level.

Breaking strength:

Table 7, 7a and figure 3 show the course of breaking strength during different periods of ambient temperature and vitamin C additions. In the cold climate there is a slight but steady decrease of breaking strength throughout the experiment. No improvement in the treated group with vitamin C can be observed.

In the hot environment no effect of the fluctuating temperature during the acclimatization period is noticed, while in the stress period a highly significant decrease in breaking strength occurs in the hot climate, though to a nearly significantly different degree for the two lots. Therefore no definite proof is obtained

TABLE 7. Egg breaking strength as affected by different environmental temperature and Vitamin C additions.

Months	Experimental periods	Breaking strength in kg & gm				L.S.D. between[1] single groups	
		Cold environment		Hot environment			
		Control	Treatment	Control	Treatment	$P < 0.05$	$P < 0.01$
1	Control	3.691	3.601	3.721	3.620	2.03	2.67
2	Acclimatization	3.622	3.384	3.563	3.610	1.99	2.62
3	Acclimatization	3.533	3.445	3.426	3.494	2.06	2.71
4	Transition	No data available					
5	Stress	3.452	3.408	2.513	2.985	2.99	3.94
6	Vitamin C add.	3.384	3.373	2.450	2.917	2.53	3.32
7	Vitamin C add.	3.338	3.251	2.294	2.824	2.72	3.58

TABLE 7a. Analysis of variance and covariance in the different periods separated and combined showing the effect of treatments, temperatures and their interactions on egg breaking strength.

Months	Experimental periods	Calculated F Value[2]			
		Kind of analysis	Treatments	Temperature	Interactions
1	Control	A.V[3]	0.84	0.06	0.00
2	Acclimatization	A.V	0.88	0.67	1.97
3	Acclimatization	A.V	0.01	0.07	0.55
4	Transition	–	–	–	–
5	Stress	A.V	1.96	19.86**	2.85(*)
		A.Co[4]	–	37.80**	–
6	Vitamin C additions	A.V	3.13(*)	28.97**	3.42(*)
		A.Co	10.83**	67.83**	3.63(*)
7	Vitamin C additions	A.V	2.54	28.10**	4.94*
		A.Co	9.18**	65.56**	5.96*
6 & 7	Vitamin C add.	A.Co	12.71**	82.18**	5.83*

Foot Notes: See table 4 a.

whether vitamin C additions has caused a significant improvement in breaking strength in the hot environment, though there are indications for some effect from the analysis of covariance. Significant and nearly significant interactions are likely to be due to inbalanced distribution of the birds between groups.

As far as high environmental temperature is concerned, our findings are supported by those of HEUSER and NORRIS (1946) who came to the conclusion that breaking strength is lower during July and August than during winter.

Table 8 shows the correlations of breaking strength with other shell quality

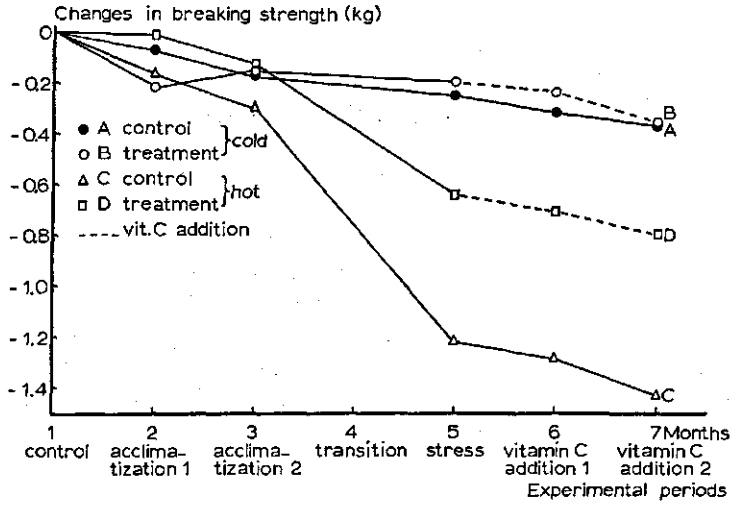


FIG. 3. Changes in breaking strength from the initial values during the course of environmental temperature changes and vitamin C additions.

TABLE 8. Correlation of breaking strength and different shell characters based on hen average, corrected for temperature and treatment effect.

Months	Experimental periods	Shell thickness				Specific gravity	Shell percentage	Deformation
		Blunt	Pointed	Equator	Mean			
1	Control	0.748**	0.571**	0.625**	0.693**	0.118	0.636**	–0.643**
2	Acclimatization	0.801**	0.670**	0.712**	0.775**	0.732**	0.775**	–0.602**
3	Acclimatization	0.699**	0.644**	0.670**	0.718**	0.700**	0.727**	–0.685**
4	Transition	–	–	–	–	–	–	–
5	Stress	0.868**	0.812**	0.804**	0.867**	0.782**	0.805**	–0.774**
6	Vitamin C add.	0.755**	0.743**	0.718**	0.781**	0.739**	0.717**	–0.669**
7	Vitamin C add.	0.641**	0.644**	0.699**	0.653**	0.616**	0.625**	–0.670**
	Correlation of breaking strength with different shell characteristics based on egg averages for month 6 and 7 corrected for temperature and treatment effect.							
6 & 7	Vitamin C add.	0.340*	0.328*	0.312*	–	0.398**	0.431**	–0.367*

* Significant at 5% level. ** Significant at 1% level.

characters on the basis of hen averages for all periods and on a basis of individual eggs for month 6 and 7 only combined. From those tables we can see that breaking strength is significantly and positively correlated with specific gravity, shell percentage, mean shell thickness and shell thickness in the three parts of the shell blunt end, pointed end and equator. The correlations on basis of individual eggs are lower than those on the basis of hen averages, supporting the conclusion about genetical influences mentioned before. Similar results concerning the high positive correlations with mentioned items were reported by many workers as MORGAN (1932), NOVIKOFF et al (1949), GODFREY (1949) and RAUCH (1959).

Shell thickness:

Tables 9 and 9a and figure 4 show the course of mean shell thickness, shell thickness of the different parts of the shell (blunt end, pointed end and equator) throughout the different periods of ambient temperature and vitamin C additions. From these data it can be seen that shell thickness behaves differently from deformation and breaking strength throughout the experiment in cold climate; shell thickness under those circumstances shows only a temporary, decline, with a recovery at the end of the experiment. If this difference in trend would be significant, which is hardly demonstrable with this number of birds, it might suggest that shell thickness is mainly associated reversely with intensity of egg production which is highest in the middle of the experiment, whereas some shell structure characters show a steady deterioration with advancing age, which is reflected in a slight but steady deterioration of deformation and breaking strength. It would also suggest that shell thickness is not a reliable characteristic for measuring shell quality throughout the year.

TABLE 9. Egg shell thickness in different parts of the egg as affected by different environmental temperature and vitamin C additions.

Months	Experimental periods	Parts of shell thickness	Cold environment		Hot environment		L.S.D. between[2] single groups	
			Control	Treatment	Control	Treatment	$P < 0.05$	0.01
1	Control	Blunt	33.25	32.58[1]	32.83	33.42	1.11	1.46
		Pointed	34.67	33.50	33.83	33.75	1.13	1.48
		Equator	33.92	33.08	33.58	33.92	1.02	1.34
		Mean	33.92	33.00	33.33	33.58	1.00	1.31
2	Acclimatization	B.	33.50	33.00	33.00	33.25	1.12	1.48
		P.	35.17	34.08	34.08	34.33	1.19	1.57
		Eq.	34.25	33.67	33.50	34.00	1.07	1.41
		Mean	34.42	33.50	33.42	33.83	1.08	1.42
3	Acclimatization	B.	33.00	32.25	32.25	32.83	1.17	1.53
		P.	34.50	33.50	33.67	34.33	1.22	1.60
		Eq.	33.33	32.50	32.75	33.75	1.11	1.46
		Mean	33.42	32.58	32.92	33.75	1.07	1.41
5	Stress	B.	32.92	32.42	28.75	30.83	1.23	1.62
		P.	34.17	33.17	30.17	32.58	1.31	1.72
		Eq.	33.67	32.83	29.42	31.83	1.18	1.55
		Mean	33.67	32.67	29.33	31.58	1.21	1.59
6	Vitamin C addition	B.	33.67	33.25	29.42	31.08	1.01	1.33
		P.	34.33	33.75	30.42	32.25	1.07	1.40
		Eq.	34.25	33.83	30.17	32.08	0.95	1.25
		Mean	33.92	33.58	29.83	31.92	1.01	1.33
7	Vitamin C addition	B.	33.25	33.08	29.42	31.33	0.89	1.17
		P.	34.00	33.67	30.25	32.67	1.04	1.37
		Eq.	33.75	33.83	30.17	32.25	0.98	1.29
		Mean	33.58	33.50	30.00	32.08	0.93	1.23

[1] Mean shell thickness in 0.01 of a millimeter.
[2] L.S.D. = Least significant difference.
Period 4: Transition period (No data available).

TABLE 9a. Analysis of variance and covariance in the different periods separated and combined showing the effect of treatment, temperature and their interactions on different parts of the egg shell thickness.

Months	Experimental periods	Different parts of shell	Calculated F Value[2]			
			Kind of analysis	Treatments	Temperature	Interactions
1	Control	Blunt	A.V[3]	0.01	0.13	1.20
		Pointed	A.V	1.17	0.26	0.88
		Equator	A.V	0.23	0.23	1.25
		Mean shell th.	A.V	0.42	0.00	1.30
2	Acclimatization	B.	A.V	0.05	0.05	0.42
		P.	A.V	0.47	0.47	1.20
		E.	A.V	0.01	0.14	0.97
		M.S.T.	A.V	0.21	0.37	1.46
3	Acclimatization	B.	A.V	0.02	0.02	1.25
		P.	A.V	0.07	0.00	1.79
		E.	A.V	0.02	0.34	2.61
		M.S.T.	A.V	0.00	0.37	2.31
5	Stress	B.	A.V	1.58	20.81**	4.20*
			A.Co[4]	–	37.58**	–
		P.	A.V	1.11	11.65**	6.48*
			A.Co	–	15.13**	–
		E.	A.V	1.72	18.86**	7.23*
			A.Co	–	36.35**	–
		M.S.T.	A.V	1.02	19.09**	6.87*
			A.Co	–	31.54**	–
6	Vitamin C additions	B.	A.V	1.44	38.07**	4.01(*)
			A.Co	2.33	68.41**	2.54
		P.	A.V	1.30	24.50**	4.88*
			A.Co	2.76	30.46**	3.22(*)
		E.	A.V	2.39	36.08**	5.77*
			A.Co	4.30*	58.87**	4.19*
		M.S.T.	A.V	2.84(*)	30.64**	5.41*
			A.Co	4.25*	44.36**	2.73
7	Vitamin C additions	B.	A.V	3.65(*)	37.15**	5.17*
			A.Co	7.01*	77.28**	4.03
		P.	A.V	3.83*	19.91**	6.67*
			A.Co	8.83**	29.96**	5.14*
		E.	A.V	4.63*	26.33**	3.95
			A.Co	9.82**	53.41**	2.42
		M.S.T.	A.V	4.37*	27.31**	5.13*
			A.Co	8.60**	51.43**	2.41
6 & 7	Vitamin C additions	B.	A.Co	5.40*	95.16**	1.69
		P.	A.Co	4.45*	41.59**	3.13(*)
		E.	A.Co	6.63*	62.29**	3.66(*)
		M.S.T.	A.Co	7.76**	71.39**	3.51(*)

Foot Notes: See table 4 a.
Period 4: Transition period (No data available).

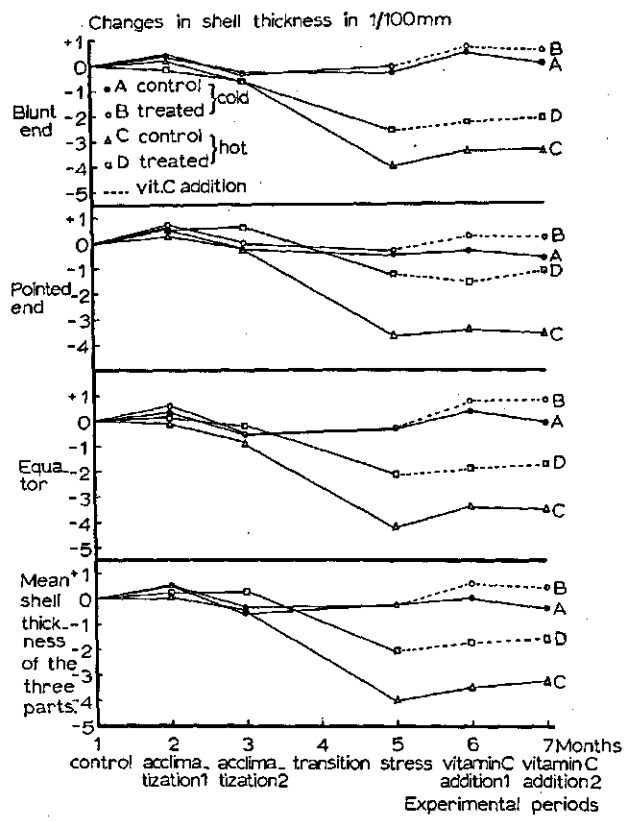


FIG. 4.
Changes in shell thickness from the initial values during the course of environmental temperature changes and vitamin C additions.

In the cold climate no significant effect of vitamin C addition on shell thickness can be observed. Because of interactions present already in the stress period there is no definite proof that vitamin C improved shell thickness in the hot period. Though the analysis of covariance suggests that vitamin C is likely to have improved shell thickness significantly in the hot climate. In fact several workers found a favorable effect of vitamin C on shell quality in hot climate, like THORNTON and MORENG (1958), THORNTON (1960), SULLIVAN and GEHLE (1962) and SULLIVAN and KINGAN (1962). They all reported that shell thickness was increased significantly by supplementation of ascorbic acid.

THORNTON and MORENG (1959) gave a conclusion about the role of vitamin C in improvent of shell thickness. They mentioned that ascorbic acid is effective in partially preventing normal decline in feed consumption and egg shell thickness in the chicken. He added that ascorbic acid may have had an influence of a stimulatory nature on the thyroid activity particularly under the conditions of increased environmental temperature.

Concerning the hot environment, we can conclude that the fluctuating (acclimatization) temperature has no significant effect on the shell thickness in different parts of the shell and as an average. This agrees with the findings of MUEL-

LER (1961) who noticed that birds kept in cycling environment produced eggs of significantly better shells than in a constant 90 °F environment. High temperature was decreasing shell thickness highly significantly in all the parts of the shell. This agrees with the findings of BENNION and WARREN (1933a), WARREN and SCHNEPEL (1940), WILHELM (1940) and BRANT et al (1953).

CONRAD (1939) gave a reasonable explanation for the diminishing of shell thickness in birds subjected to high temperatures. He mentioned that shell secretion is probably retarded at high temperatures by the diminished intake of calcium in the feed, and possibly also by a reduced capacity of the blood stream to carry calcium.

Table 10 and 10a show the correlations between the thickness of different parts of the shell and the different other shell characteristics. It is clear from the tables, that there was a highly positive significant correlation of shell thickness with the other shell characteristics except deformation which was also highly significant but naturally negative. These conclusions agree with the findings of

TABLE 10. Correlations of shell thickness in different parts of the shell and different shell characteristics based on hen averages and corrected for temperature and treatment effect.

Months	Experimental Periods	Items of shell thickness	Blunt shell thickness	Pointed shell thickness	Equator shell thickness	Deformation	Breaking strength	Specific gravity	Shell percentage
1	Control	Mean shell thickness	0.914**	0.911**	0.930**	-0.875**	0.693**	0.345*	0.719**
		Blunt		0.797**	0.823**	-0.792**	0.748**	0.349*	0.682**
		Pointed			0.781**	-0.745**	0.571**	0.350*	0.605**
		Equator				-0.886**	0.625**	0.401**	0.695**
2	Acclimatization	Mean	0.936**	0.929**	0.932**	-0.739**	0.775**	0.897**	0.866**
		Blunt		0.831**	0.875**	-0.652**	0.801**	0.839**	0.824**
		Pointed			0.826**	-0.694**	0.670**	0.782**	0.731**
		Equator				-0.835**	0.712**	0.915**	0.945**
3	Acclimatization	Mean	0.937**	0.939**	0.911**	-0.836**	0.718**	0.888**	0.851**
		Blunt		0.861**	0.837**	-0.731**	0.699**	0.796**	0.796**
		Pointed			0.781**	-0.741**	0.644**	0.787**	0.723**
		Equator				-0.904**	0.670**	0.912**	0.922**
5	Stress	Mean	0.938**	0.940**	0.953**	-0.848**	0.867**	0.894**	0.932**
		Blunt		0.882**	0.887**	-0.793**	0.868**	0.851**	0.855**
		Pointed			0.865**	-0.771**	0.812**	0.833**	0.870**
		Equator				-0.851**	0.804**	0.908**	0.946**
6	Vitamin C addition	Mean	0.904**	0.941**	0.930**	-0.825**	0.781**	0.873**	0.823**
		Blunt		0.797**	0.845**	-0.675**	0.755**	0.765**	0.707**
		Pointed			0.887**	-0.815**	0.743**	0.821**	0.791**
		Equator				-0.846**	0.719**	0.901**	0.876**
7	Vitamin C addition	Mean	0.929**	0.917**	0.948**	-0.712**	0.653**	0.822**	0.835**
		Blunt		0.819**	0.904**	-0.682**	0.641**	0.768**	0.783**
		Pointed			0.889**	-0.698**	0.644**	0.730**	0.781**
		Equator				-0.785**	0.699**	0.855**	0.896**

* Significant at 5% level.
** Significant at 1% level.
Period 4: Transition period (No data available).

TABLE 10a. Correlation coefficients of shell thickness in different parts of the shell and different shell characteristics based on egg averages for month 6 & 7 (corrected for temperature and treatment effect.

Month	Experimental periods	Items of shell thickness	Pointed shell thickness	Equator shell thickness	Deformation	Breaking strength	Specific gravity	Shell %
6 & 7	Vitamin C additions	Blunt	0.538**	0.667**	–0.456**	0.340*	0.493**	0.353*
		Pointed		0.617**	–0.394**	0.328*	0.495**	0.297*
		Equator			–0.554**	0.312*	0.632**	0.380**

* Significant at 5% level.
** Significant at 1% level.

MORGAN (1932), NOVIKOFF et al (1949), GODFREY (1949), RAUCH (1959) and concerning the deformation with SCHOORL and BOERSMA (1962). Again correlations on basis of hen averages are considerably higher than those based on individual eggs. The latter depend merely on structural relations between egg characters, whereas correlations on a hen basis are partly due to genetic variation.

Specific gravity:

Table 11, 11a and figure 5 show the course of specific gravity in the different periods of ambient temperature and vitamin C additions. In studying the figures it should be kept in mind that because of difficulties with the analysis in the first control period, measurements of specific gravity in this period are not very reliable. This may account for the low correlations between specific gravity and other shell characters (Table 12) in this period, as compared with the rest of the experiment and for the large differences between groups in this period. If this period is excluded, specific gravity like other shell characters except shell thickness shows a steady deterioration of shell quality throughout the experiment in

TABLE 11. Specific gravity of eggs as affected by environmental temperature and vitamin C additions.

Months	Experimental	Specific gravity				L.S.D. between[1] single groups	
		Cold environment		Hot environment			
		Control	Treatment	Control	Treatment	P< 0.05	– 0.01
1	Control	1.0846	1.0860	1.0865	1.0874	0.0105	0.0137
2	Acclimatization	1.0862	1.0854	1.0852	1.0857	0.0211	0.0277
3	Acclimatization	1.0854	1.0846	1.0843	1.0854	0.0207	0.0272
4	Transition	No data available					
5	Stress	1.0831	1.0823	1.0755	1.0789	0.0248	0.0373
6	Vitamin C add.	1.0821	1.0816	1.0763	1.0799	0.0238	0.0312
7	Vitamin C add.	1.0813	1.0805	1.0760	1.0795	0.0220	0.0289

TABLE 11a. Analysis of variance and covariance in the different periods separated and combined showing the effect of treatment, temperature and their interactions on specific gravity of the egg.

Months	Experimental periods	Calculated F. Value[2]			
		Kind of analysis	Treatment	Temperature	Interactions
1	Control	A.V[3]	0.98	1.11	0.60
2	Acclimatization	A.V	0.05	0.15	0.49
3	Acclimatization	A.V	0.02	0.02	0.89
4	Transition	–	–	–	–
5	Stress	A.V	0.79	14.59**	1.98
		A.Co	–	15.47**	–
6	Vitamin C	A.V	1.98	9.74**	3.06(*)
	additions	A.Co[4]	1.66	9.98**	3.14(*)
7	Vitamin C	A.V	1.68	7.30**	4.45*
	additions	A.Co	1.27	8.01**	4.70*
6 & 7	Vitamin C add.	A.Co	1.86	10.85**	4.06*

Foot notes; See table 4 a.

the cold environment, due to the normal decline of shell quality caused by the age of birds. No effect was noticed of vitamin C additions on specific gravity in the cold climate.

Concerning the hot environment, it was noticed that the fluctuating temperature had no significant effect on shell quality as measured by specific gravity. After increasing the temperature in the stress period, a highly significant drop in specific gravity was seen. No significant effect of vitamin C additions could be shown in the hot environment either.

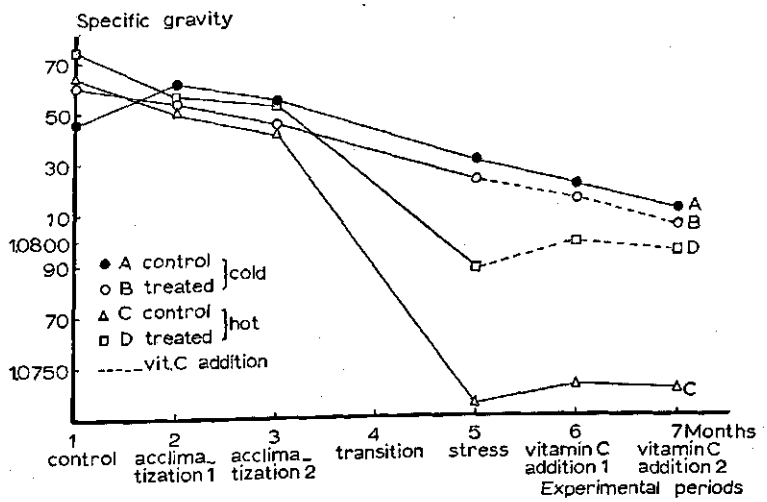


FIG. 5. Specific gravity of eggs during the course of environmental temperature changes and vitamin C additions.

Specific gravity is not a very reliable measurement for shell quality unless precautions are taken to prevent weight loss of the eggs by evaporation prior to the determination of specific gravity.

A lot of precautions were taken in this case to avoid such losses. It seems also that specific gravity is not a very sensitive measure to test a small improvement in shell quality, since the other shell quality determinations showed more important effects of vitamin C additions than did specific gravity. Similar findings were reported by ARSCOTT et al (1962) who noticed that there was no improvement in egg shell quality as measured by specific gravity in Leghorn Layers fed rations containing ascorbic acid. Interactions noticed are likely to be due to an unequal distribution of birds in groups.

From table 12 it was observed that specific gravity was highly positively correlated with breaking strength, mean shell thickness and shell percentage and negatively with deformation. The significant correlations were noticed in all the experimental periods except the control period in which some insignificant correlations also were observed. It was observed again that the correlations based on egg averages were lower than those based on hen averages indicating genetic effects in the latter.

TABLE 12. Correlations of specific gravity with different shell characters based on hen average corrected for temperature and treatment effect.

Months	Experimental periods	Breaking strength	Deformation	Mean shell thickness	Shell percentage
1	Control	0.118	-0.428**	0.345*	0.204
2	Acclimatization	0.732**	-0.786**	0.897**	0.916**
3	Acclimatization	0.700**	-0.860**	0.888**	0.923**
4	Transition	–	–	–	–
5	Stress	0.782**	-0.864**	0.894**	0.926**
6	Vitamin C add.	0.739**	-0.915**	0.873**	0.945**
7	Vitamin C add.	0.616**	-0.819**	0.822**	0.900**
	Correlation of specific gravity with different shell characters based on egg averages for month 6 and 7 corrected for temperature and treatment effect				
6 & 7	Vitamin C add.	0.398**	-0.594**	0.632** ⊙	0.453**

* Significant at 5% level.
** Significant at 1% level.
⊙ Equator shell thickness was used instead of mean shell thickness.

Shell percentage:

Table 13 and 13a and figure 6 show the course of shell percentage during the different periods of ambient temperature and vitamin C additions.

From the figure we can notice that the shell percentage curve did not show any fluctuations in the cold environment except a tendency for lowering throughout the experiment in both treated and control groups in the cold climate. This drop in shell percentage is naturally to be ascribed to the age of birds. No effect

TABLE 13. Egg shell percentage as affected by environmental temperature and vitamin C additions.

Months	Experimental periods	Egg shell percentage				L.S.D. between[1] single groups	
		Cold environment		Hot environment			
		Control	Treatment	Control	Treatment	$P < 0.05$	$P < 0.01$
1	Control	9.55	9.26	9.26	9.35	0.61	0.87
2	Acclimatization	9.23	9.12	9.10	9.12	0.46	0.61
3	Acclimatization	8.97	8.95	8.95	9.12	0.46	0.64
4	Transition	No data available					
5	Stress	8.80	8.71	7.81	7.95	0.62	0.82
6	Vitamin C add.	8.66	8.71	7.85	8.40	0.58	0.76
7	Vitamin C add.	8.56	8.55	7.90	8.40	0.58	0.76

TABLE 13a. Analysis of variance and covariance in the different periods separated and combined showing the effect of treatment, temperature and their interactions on egg shell percentage.

Months	Experimental periods	Calculated F. Value[2]			
		Kind of analysis	Treatments	Temperature	Interactions
1	Control	A.V[3]	0.44	0.17	1.02
2	Acclimatization	A.V	0.01	0.22	0.63
3	Acclimatization	A.V	0.10	0.27	0.78
4	Transition	–	–	–	–
5	Stress	A.V	1.52	14.28**	3.33(*)
		A.Co[4]	–	25.97**	–
6	Vitamin C additions	A.V	2.90(*)	10.91**	1.96
		A.Co	3.82(*)	13.59**	0.95
7	Vitamin C additions	A.V	2.32	5.88*	2.67
		A.Co	3.84(*)	9.96**	1.41
6 & 7	Vitamin C add.	A.Co	4.39*	13.33**	1.25

Foot notes: See table 4 a.

was noticed of the vitamin C additions on shell percentage in the cold environment.

Concerning the hot environment, it was noticed that the fluctuating temperature had no significant effect on shell quality as measured by shell percentage. Concerning the high temperature in the stress period, a highly significant temperature effect on shell percentage was observed with a clear drop in shell percentage. These findings agree with the work of BENNION and WARREN (1933a), WILHELM (1940) and MUELLER (1961).

As far as vitamin C is concerned for improving shell quality as measured by shell percentage in hot climate, a marked increase was noticed from the curve

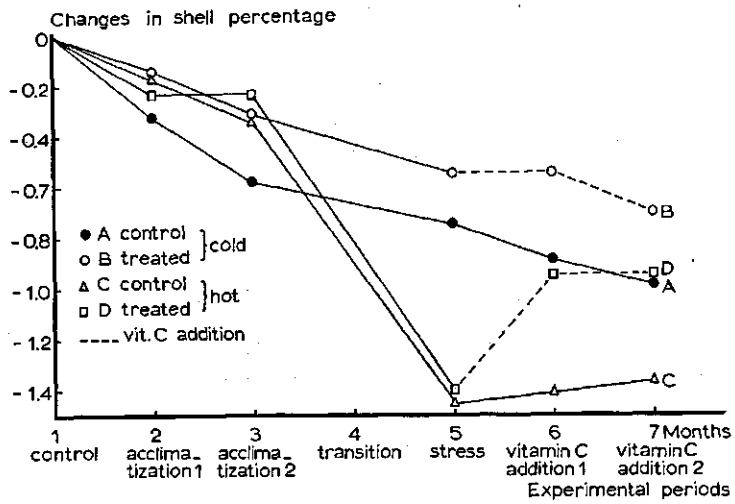


FIG. 6. Changes in egg shell percentage during the course of environmental temperature changes and vitamin C additions.

in the shell percentage of the treated stressed birds. This remarkable improvement was statistically nearly significant in each of the two treatment months separately ($P < 0.05$) and significant if the two months were combined ($P < 0.05$). This observation agrees with the findings of THORNTON and MORENG (1958–1959).

Table 14 shows the correlations of shell percentage with different shell characters. It was observed that shell percentage was highly positively correlated with breaking strength, mean shell thickness and specific gravity and negatively with

TABLE 14. Correlations of shell percentage with different shell characters based on hen average corrected for temperature and treatment effect.

Months	Experimental periods	Breaking strength	Deformation	Mean shell thickness	Specific gravity
1	Control	0.636**	–0.697**	0.719**	0.204
2	Acclimatization	0.775**	–0.856**	0.866**	0.916**
3	Acclimatization	0.727**	–0.912**	0.851**	0.923**
4	Transition	–	–	–	–
5	Stress	0.805**	–0.899**	0.932**	0.926**
6	Vitamin C add.	0.717**	–0.926**	0.823**	0.945**
7	Vitamin C add.	0.625**	–0.894**	0.835**	0.900**
	Correlation of shell percentage with different shell characters based on hen average corrected for temperature and treatment effect				
6 & 7	Vitamin C add.	0.431**	–0.362*	0.380** ⊙	0.453**

* Significant at 5% level.
** Significant at 1% level.
⊙ Equator shell thickness was used instead of mean shell thickness.

deformation. The same observation of specific gravity in the control period was noticed also, the hen average correlation was higher than the egg average correlation due to genetic influences.

Shape index:

Table 15 and 15a and fig. 7 show the course of egg shape index in the different periods of ambient temperature and vitamin C additions. No clear trend in egg shape index was observed in the cold climate though there are indications of a slight decrease of the index during the first 5 months. This decrease is likely to be associated with the increase in egg weight occurring in the same period.

TABLE 15. Egg shape index as affected by environmental temperature and vitamin C additions.

Months	Experimental periods	Egg shape index				L.S.D. between[1] single groups	
		Cold environment		Hot environment			
		Control	Treatment	Control	Treatment	$P < 0.05$	$P < 0.01$
1	Control	75.00	74.08	74.58	74.58	1.32	1.74
2	Acclimatization	74.33	73.42	73.92	73.25	1.57	2.07
3	Acclimatization	74.08	73.67	73.92	73.75	1.41	1.85
4	Transition			No data available			
5	Stress	73.75	73.33	73.00	73.00	1.25	1.65
6	Vitamin C add.	73.83	73.83	72.17	73.25	1.38	1.81
7	Vitamin C add.	73.83	74.42	72.67	73.42	1.35	1.78

TABLE 15a. Analysis of variance and covariance in the different periods separated and combined showing the effect of treatment, temperature and their interactions on egg shape index.

Months	Experimental periods	Calculated F. Value[2]			
		Kind of analysis	Treatments	Temperature	Interactions
1	Control	A.V[3]	0.46	0.00	0.46
2	Acclimatization	A.V	0.97	0.13	0.02
3	Acclimatization	A.V	0.16	0.00	0.03
4	Transition	–	–	–	–
5	Stress	A.V	0.11	0.71	0.11
		A.Co[4]	–	1.51	–
6	Vitamin C add.	A.V	0.59	2.54	0.59
		A.Co	2.84(*)	5.49*	0.49
7	Vitamin C add.	A.V	0.92	2.44	0.01
		A.Co	4.33*	5.77*	0.07
6 & 7	Vitamin C add.	A.Co	3.33(*)	6.51*	0.08

Foot notes: See table 4 a.

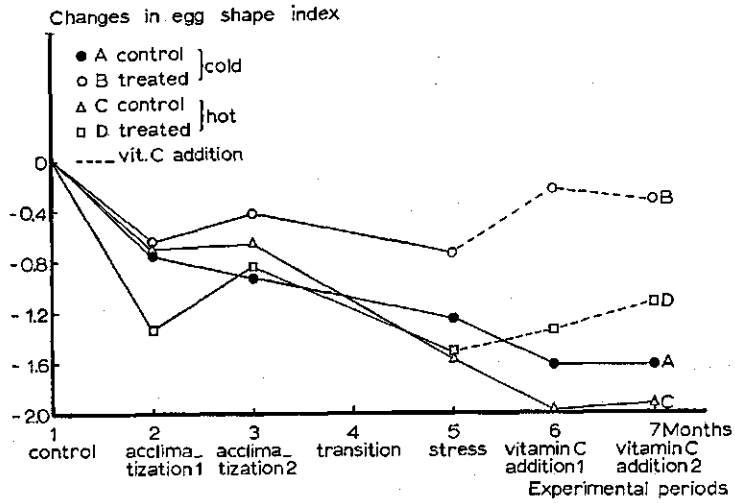


FIG. 7. Changes in egg shape index from the initial values during the course of environmental temperature changes and vitamin C additions.

In the groups of hot environment, a similar decrease in egg shape index was observed during the acclimatization and stress period, but no effect of temperature on egg shape was noticed even in the stress period. In the period of the vitamin C additions it was noticed in the group without vitamin C in hot environment that there was a tendency for a progressive decrease in shape index.

In relation to the decreasing egg size this means that the breadth of the eggs decreased to a larger extent than the width. This decrease in egg shape index as compared with the control period was significant ($P < 0.05$) according to the analysis of covariance. It is generally believed that the pressure exerted on the egg in the oviduct determines its shape. Opinions differ, however, as to what part of the oviduct influences shape index most (STURKIE, 1965).

Egg shape is influenced most by the isthmus, according to some, and by the uterus, according to others.

These findings seem to agree with the work of BENJAMIN (1920) and the reports of the STICHTING VOOR HET FOKKERIJWEZEN BIJ DE PLUIMVEEHOUDERIJ, ZEIST (1962–1963). They reported that summer eggs are lower in shape index than winter eggs.

However, the results are different in a sense because summer eggs in moderate climate not only have a lower shape index but are also larger than winter eggs. Here in the hot climate the eggs are smaller and still have a lower shape index. It is well known that birds under heat stress always shows a high respiration rate and panting. This high rate of respiration is causing different abdominal muscular contractions. It may be that these contractions are inducing some uteral convulsions which are the cause of the narrow long eggs of low shape index in the hot weather. It may be suggested also that during the heat stress there is more evaporation, an increased blood flow through the lungs and less water

used for egg formation causing smaller eggs which will be narrower in the first place if length of the egg is primarily determined by the isthmus.

According to the analysis of covariance, vitamin C addition increased egg shape index significantly ($P < 0.05$) or nearly significant, both in the cold and in the hot environment. In the cold environment this might be rather effected by the much lower egg weight, presumably caused by uneven distribution of birds. In the hot environment however, vitamin C tended to increase egg weight, so in this case the effect of vitamin C is likely to be related to an alleviation of the heat stress.

Formation period:

Table 16, 16a and figure 8 show the intervals between subsequent ovipositions in a clutch, expressed in hours and centihours as they are affected by different environmental temperature and vitamin C additions. These intervals are closely related to the egg formation period.

From the figure we can see that in the cold environment oviposition intervals tended to increase in both treated and untreated groups with advancing laying season. As far as vitamin C additions are concerned, no significant effect was noticed.

The fluctuating temperature showed no significant increase on oviposition intervals. The high temperature however showed a highly significant increase in oviposition intervals. Vitamin C additions did not significantly effect oviposition intervals in the hot environment either.

BERG (1945) reported that increases or decreases in the time interval between eggs within a clutch are accompanied by corresponding increases or decreases in shell thickness. From our work in this experiment it was clearly noticed that high temperature brought about a highly significant decrease in shell thickness. It might be suggested that this reduction in shell thickness would have been caused by a premature expelling. Since, however, oviposition intervals were

TABLE 16. Oviposition intervals in hours as affected by environmental temperature and vitamin C additions.

Months	Experimental periods	Oviposition intervals in hours				L.S.D. between[1] single groups	
		Cold environment		Hot environment			
		Control	Treatment	Control	Treatment	$P < 0.05$	$P < 0.01$
1	Control	25.33	25.08	25.50	25.17	0.58	0.76
2	Acclimatization	25.50	25.42	25.25	25.50	0.56	0.73
3	Acclimatization	26.07	25.50	26.07	26.32	0.54	0.71
4	Transition			No data available			
5	Stress	26.25	26.00	27.08	28.00	0.68	0.90
6	Vitamin C add.	27.15	28.17	28.15	28.58	0.69	0.91
7	Vitamin C add.	27.07	26.25	29.32	29.15	0.66	0.86

TABLE 16a. Analysis of variance and covariance in the different periods separated and combined showing the effect of treatment, temperature and their interactions on oviposition intervals in hours.

Months	Experimental periods	Kind of analysis	Calculated F. Value[2] Treatments	Temperature	Interactions
1	Control	A.V[3]	0.97	0.18	0.02
2	Acclimatization	A.V	0.08	0.08	0.34
3	Acclimatization	A.V	0.02	0.56	0.56
4	Transition	–	–	–	–
5	Stress	A.V	0.90	16.26**	2.76
		A.Co[4]	–	14.76**	–
6	Vitamin C additions	A.V	0.12	22.97**	3.95(*)
		A.Co	0.14	24.00**	4.69*
7	Vitamin C additions	A.V	0.75	49.57**	0.14
		A.Co	1.01	68.09**	1.01
6 & 7	Vitamin C add.	A.Co	0.02	61.78**	0.02

Foot notes: See table 4 a.

significantly increased, the contrary seems to be true and though the ovulation-oviposition intervals are not known, the time of egg (shell) formation is likely to be increased, rather than shortened.

This increase in formation time of the egg shell may serve as a natural protection against a decrease in shell thickness, occuring during hot weather. Without this increase in formation period, shell thickness most likely would have decreased even more in the hot climate.

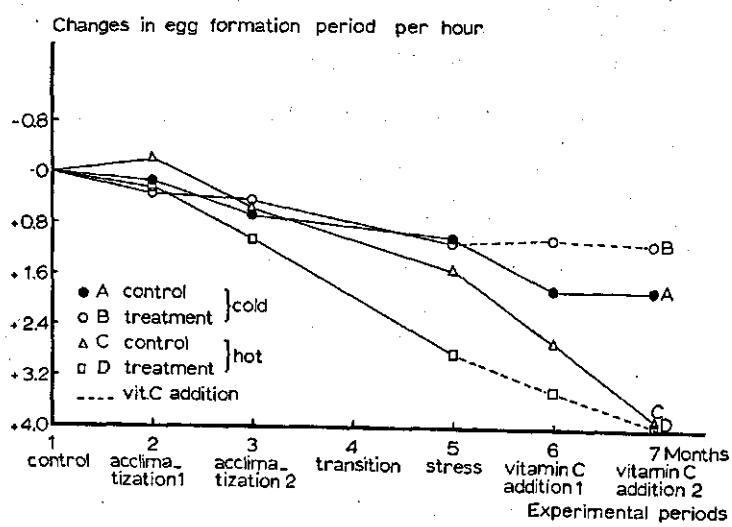


FIG. 8. Changes in formation period of eggs per hour from the initial values during the course of environmental temperature changes and vitamin C additions.

TABLE 17. Means of egg shell deformation and breaking strength in clutches of different lengthes as affected by cold and hot environment, during the stress period.

Clutch size	Tempe-rature	No of clutches	Position of eggs in clutch											
			1		2		3		4		5		6		7	
			Def.[1]	B.S.[2]	Def.	B.S.	Def.	B.S.	Def.	B.S.	Def.	B.S.	Def.	B.S.	Def.	B.S.
2	Cold	72	28.3	3.312	29.9	3.059										
	Hot	39	33.8	2.887	34.2	2.786										
3	Cold	39	26.2	3.625	27.7	3.505	25.7	3.759								
	Hot	25	33.6	3.003	35.3	2.842	33.1	2.612								
4	Cold	25	28.3	3.446	30.1	3.307	28.7	3.372	26.9	3.288						
	Hot	12	34.3	2.663	33.3	2.959	33.0	2.862	33.1	2.854						
5	Cold	28	27.7	3.614	28.4	3.462	29.7	3.088	29.0	3.121	26.7	3.403				
	Hot	1	33.0	2.405	34.0	2.540	30.0	2.005	30.0	1.600	30.0	2.190				
6	Cold	2	27.5	3.398	27.0	3.165	28.8	3.500	26.8	3.339	28.1	3.169	25.1	3.888		
	Hot	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7	Cold	3	28.8	3.393	32.1	2.651	31.3	3.000	33.6	2.999	34.4	2.554	36.3	2.850	30.1	3.434
	Hot	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—

[1]: Deformation in μ under a load of 500 g.
[2]: Breaking strength in kg and grams.

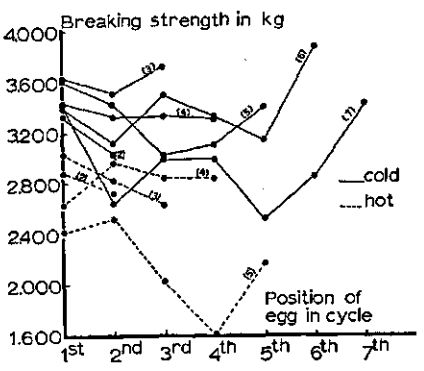


FIG. 9. Means of breaking strength and clutches in different lengths as affected by cold and hot environment.

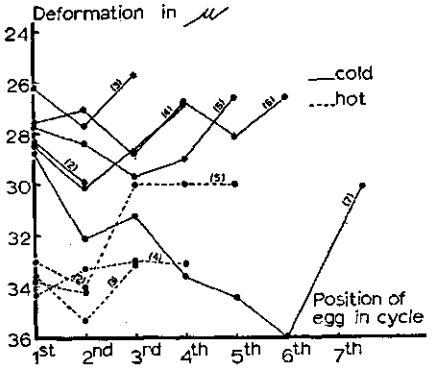


FIG. 10. Means of deformation and clutches in different lengths as affected by cold and hot environment.

Clutch size and position of egg in the clutch:

Table 17 shows the relation between clutch size, position of egg in clutch and shell quality determinations as breaking strength in kg and deformation in μ as affected by cold and hot environment during the stress period.

From the table we can see that less eggs were produced in the hot environment and the clutch size did not reach more than five eggs. The data presented in table 17 concerning the effect of clutch size and position in the clutch on shell quality are illustrated in figure 9 and 10.

In the cold environment there was a tendency towards a decrease in breaking strength and an increase in deformation in the second egg of a clutch as compared with the first egg.

Moreover there was a tendency towards an increase in breaking strength and a decrease in deformation in the last eggs of clutches of 3 or more eggs. These findings agree with the work of TAYLOR and LERNER (1939). They reported that the second egg of a clutch has a thinner shell than the first egg. WILHELM (1940) reported that there is a little decrease in shell thickness between the first and last eggs of the same clutch of 1, 2, 3 and 4-egg clutches. BERG (1945) came to the conclusion that shells of the first and last egg of a clutch of three or more eggs were usually thicker than the intervening eggs. This is confirmed by our data.

Concerning the hot climate, it is clear from the table and the figures that the hot environment showed in general low estimates of breaking strength and high estimates of deformation compared with eggs of cold climate.

In the 2 and 3-egg clutches, shell deformation shows similar differences between subsequent eggs in the clutch as in the cold environment. However breaking strength is lowered in the last egg of the 3-egg clutch, contradictory to what is seen in the cold climate and to the raise of deformation in the hot environment. In the clutches of 4 and 5 eggs, no clear trend is seen in shell quality in the hot climate, probably due to the small number of observations. We can con-

clude from these results that the hot climate is affecting and disturbing the normal secretion of the shell, thus causing irregularities in the sequence of the successive eggs. The exact explanation to this case needs further investigation.

Plasma blood calcium and shell quality:

Table 18, 18a and figure 11 show the changes in total blood plasma calcium in different periods of environmental temperature and vitamin C additions. From the figure we can see that the normal trend in the cold climate does not show fluctuations in total plasma calcium from the control through the last vita-

TABLE 18. Total plasma blood calcium as affected by environmental temperature and vitamin C additions.

Months	Experimental periods	Plasma blood calcium mg %				L.S.D. between[1] single groups	
		Cold environment		Hot environment			
		Control	Treatment	Control	Treatment	$P < 0.05$	$P < 0.01$
1	Control	26.15	26.15	27.00	26.35	1.93	2.54
2	Acclimatization	27.50	26.50	26.80	25.40	2.14	2.82
3	Acclimatization	27.50	26.35	26.80	25.30	2.14	2.81
4	Transition			No data available			
5	Stress	27.25	27.15	23.01	21.35	2.26	3.00
6	Vitamin C add.	27.70	28.25	21.75	23.60	2.70	3.55
7	Vitamin C add.	27.25	27.05	20.50	23.35	2.44	3.21

TABLE 18a. Analysis of variance and covariance in the different periods separated and combined showing the effect of treatment, temperature and interactions on plasma blood calcium mg %.

Months	Experimental periods	Calculated F. Value[2]			
		Kind of analysis	Treatments	Temperature	Interactions
1	Control	A.V[3]	0.13	0.28	0.15
2	Acclimatization	A.V	1.90	0.87	0.06
3	Acclimatization	A.V	1.95	0.85	0.05
4	Transition	–	–	–	–
5	Stress	A.V	2.13	36.77**	0.03
		A.Co[4]	–	35.05**	–
6	Vitamin C additions	A.V	1.04	19.45**	0.34
		A.Co	2.37	19.71**	0.55
7	Vitamin C additions	A.V	1.75	24 53**	2.17
		A.Co	2.92(*)	24 24**	2.16
6 & 7	Vitamin C add.	A.Co	4.49*	38 10**	2.96(*)

Foot notes: See table 4 a.

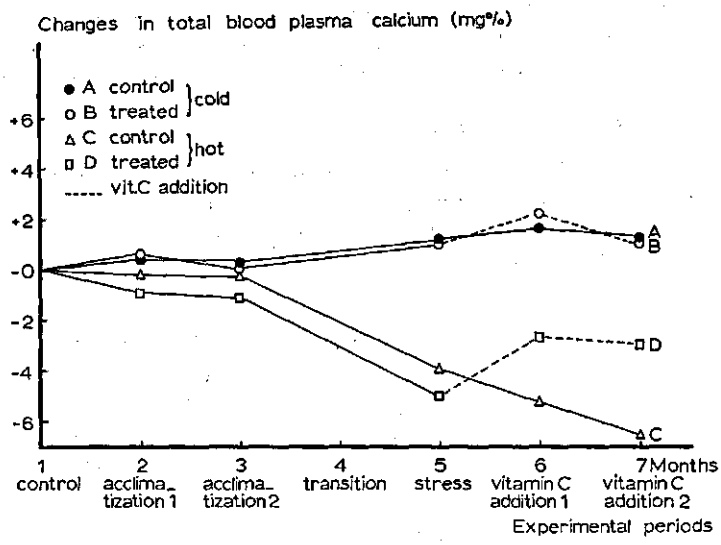


FIG. 11. Changes in total blood plasma calcium during the course of environmental temperature changes and vitamin C additions.

min C addition period. There was no marked heightening effect of vitamin C additions on the calcium level in the plasma.

Concerning the effect of hot environment, it was noticed that the fluctuating (acclimatization) temperature, had no significant effect on total calcium in blood plasma of which samples were taken during the (hot) day period.

MULLER (1959) came to similar conclusions. He reported that serum calcium levels in the variable environment were significantly higher than those in the constantly hot environments.

The stress period showed a significant effect of high temperature on calcium in blood plasma, this level being greatly reduced by high temperature and humidity. This significant lowering in plasma calcium was accompanied by a significant reduction in shell quality. Concerning this effect, BENNION and WARREN (1933a) and WARREN and SCHNEPEL (1940) reported that shell thickness and blood calcium are reduced markedly if hens are subjected to high environmental temperatures. CONRAD (1939) also found that blood calcium levels were reduced at high environmental temperatures. He added that an increase in temperature from 70°F to about 90°F caused a relative decrease of 25.30% in the blood calcium level.

As far as the vitamin C additions to the feed are concerned, it is clear from the results that vitamin C raised the calcium level in the plasma in the hot environment significantly and this raise in the plasma calcium was accompanied by a significant improvement in shell quality. SULLIVAN and GEHLE (1962) reported that ascorbic acid slightly decreases calcium levels in the blood. Therefore it was thought that ascorbic acid may increase egg shell thickness by promoting the transfer of serum calcium to the shell gland of the oviduct. The work of SULLI-

TABLE 19. Shows the relation between different shell characters, total plasma blood calcium in different environmental temperature and vitamin C additions.

Experimental periods	COLD										HOT									
	CONTROL					TREATED					CONTROL					TREATED				
	S.T.[1]	B.S.[2]	S.G.[3]	Def.[4]	B. Ca.[5]	S.T.	B.S.	S.G.	Def.	B. Ca.	S.T.	B.S.	S.G.	Def.	B. Ca.	S.T.	B.S.	S.G.	Def.	B. Ca.
1 Control	33.9	3.691	1.0846	21.2	26.15	33.0	3.601	1.0860	21.4	26.15	33.3	3.721	1.0865	21.3	27.00	33.6	3.620	1.0874	21.3	26.35
2 Acclimatization	34.4	3.622	1.0862	22.8	27.50	33.5	3.384	1.0854	23.1	26.50	33.4	3.563	1.0852	22.7	26.80	33.8	3.610	1.0857	22.2	25.40
3 Acclimatization	33.4	3.533	1.0854	22.7	27.50	32.6	3.445	1.0846	24.4	26.35	32.9	3.462	1.0843	24.1	26.80	33.8	3.494	1.0854	22.6	25.30
5 Stress	33.7	3.452	1.0831	28.1	27.25	32.7	3.408	1.0823	29.2	27.15	29.3	2.513	1.0755	37.0	23.01	31.6	2.985	1.0789	32.9	21.35
6 Vitamin C additions	33.9	3.384	1.0821	30.4	27.70	33.6	3.373	1.0816	30.8	28.25	29.8	2.450	1.0763	39.6	21.75	31.9	2.917	1.0799	33.6	23.6
7 Vitamin C additions	33.6	3.338	1.0813	32.3	27.25	33.5	3.251	1.0805	33.0	27.05	30.0	2.294	1.0760	39.7	20.50	32.1	2.824	1.0795	33.8	23.35

[1]: Shell thickness in 1/100 of mm.
[2]: Breaking strength in kg and grams.
[3]: Specific gravity.
[4]: Deformation in 0.001 (μ).
[5]: Total plasma blood calcium mg %.
Period 4 is a transition period no data available.

VAN and GEHLE is contradictory to our results, showing a significant rise in serum blood calcium in the hot environment when vitamin C is added.

Since the blood calcium is highly correlated with the shell quality, it should be clear that every raise in the blood calcium should be reflected in a corresponding improvement in shell quality. A bird put in a high temperature showing a low blood calcium level with a low shell quality, will show a high blood calcium with a high shell quality when that bird is moved to a better (lower) temperature. (BENNION and WARREN, 1933a) and (WARREN and SCHNEPEL, 1940).

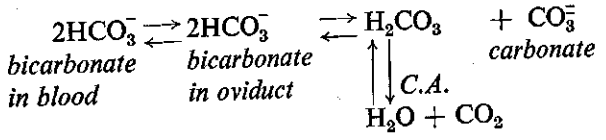
Finally, table 19 shows the relation between different shell characters and total plasma blood calcium in the different periods of the trials. It was clearly observed that shell quality determinations were lowered when total calcium blood plasma was lowered. This was clear in the hot climate. Vitamin C additions improved the shell quality and increased the calcium in blood plasma.

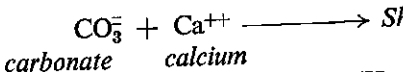
CHAPTER 3

ACID BASE BALANCE AND EXTERNAL EGG QUALITY

3.1. Introduction

Any procedure which might stimulate and increase the process of calcium deposition in the shell will be of great importance. It is an established fact that the enzyme carbonic anhydrase is involved in the calcification of the shell in the way of catalysing the decomposition of hydrocarbonic acid into carbon dioxide and water in the secreting cells of the uterus.





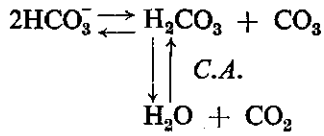
(Hertelendy and Taylor, 1961)

It was suggested that the bicarbonate concentration in the blood might be increased by increasing the alkali reserve of the blood plasma by giving additional Na^+ in organic compounds of which the acid anion rest might be broken down to CO_2 and H_2O. By increasing this bicarbonate content more $CO_3^=$ might become available, which might improve $CaCO_3$ deposition in the shell, provided that sufficient calcium is available and carbonic anhydrase activity is sufficient too. For this reason this experiment was carried on trying to increase $CaCO_3$ deposition in the shell and to investigate the effect of organic sodium compounds on shell quality and blood constituents.

3.2. Review of literature

Gutowska and Mitchell (1945) found the carbonic anhydrase content of the uterus, but not that of the blood, much higher in high laying hens which laid eggs with good shell texture, than in poor layers and nonlayers. Injection of birds with sulfanilamide caused them to lay soft shelled eggs, not because the calcium content of the blood was changed (this was not affected), but because the carbonic anhydrase in the shell gland was inactivated. These workers consider the enzyme necessary for shell formation.

According to Gutowska and Mitchell (1945), carbonic anhydrase (*C.A.*) could catalyze the decomposition of H_2CO_3 in the secreting cells of the uterus, in the reaction as follows:



The bicarbonate ions in the uterus are obtained from the blood and could react to form carbonic acid and carbonic ions. This is an equilibrium reaction in which the accumulation of carbonic acid would limit the formation of the necessary number of carbonate ions needed for the $CaCO_3$ of the shell. These workers propose that the uterus absorbs calcium proteinate from the blood and dissociates it into carbonate ions in accordance with the principle of DONNAN's equilibrium.

From the work of BENESCH et al (1944) and GUTOWSKA and MITCHELL (1945) it has been generally assumed that the enzyme carbonic anhydrase plays an important role in egg shell formation by supplying the carbonate anion of calcium carbonate through the reaction $CO_2 + H_2O \rightleftarrows H_2CO_3$.

This assumption is based on (a) the significantly greater carbonic anhydrase activity of uterine epithelium as compared with oviduct tissue and (b) the reduction of shell deposition after feeding sulfanilamide. From this assumption we can come to a suggestion that if the enzyme carbonic anhydrase can be activated, the calcium deposition of the egg will be better and the shell thickness will be higher.

HALL and HELBACKA (1959) reported that during feeding of NH_4Cl, shell thickness dropped along with a drop in blood PH. This would indicate that the laying hens were suffering from acidosis which may account for the thin shells. HUNT and AITKEN (1962) confirmed this report and suggested that plasma bicarbonate was depressed during feeding of NH_4Cl causing thinner shells.

HELBACKA et al (1963) reported from their trials that changing the acid-base balance via the respiratory system would have the same effect as feeding NH_4Cl. Finally he came to the conclusion that exposure of laying hens to an environment of high CO_2 caused a rise in Haugh units, a drop in blood pH and a reduction in shell thickness. This preliminary investigation indicates that further studies are necessary to assess the role of acid-base balance on albumen- and shell quality.

On the other hand MUELLER (1962) reported from his experiments on carbonic anhydrase, diuretics and egg shell formation the following:

a. There was no significant correlation between shell thickness and carbonic anhydrase activity of the uterine tissue.
b. The carbonic anhydrase content of the uterine tissue during shell deposition was not higher than when an egg was present in either the magnum or the isthmus.
c. Mercuhydrin, cardalin and acetazolamide interfered with shell deposition and caused diuresis.

Neohydrin had no effect on shell deposition or urine excretion. It was concluded that the interference of carbonic anhydrase inhibitors with shell formation might

be due to their diuretic effect and not to a reduction of the carbonate ion supply.

Concerning the chemical constituents of chicken blood, a lot of workers determined calcium, phosphorus, sodium, alkali reserve and pH. The following shows the chemical constituents of chicken blood as reported in the literature (from SHIMERS, 1937), with omissions and additions, expressed as milligrams per 100 ml of whole blood unless otherwise stated.

Total Calcium serum		Phosphorus		NaCl	K a-whole blood b-serum		Alkali reserve volume %	PH	Author	
Non laying	laying	Total plasma	In-organic serum							
–	–	13.0	4.60	563.0	164(a),	22(b)	42.0	7.36	Dyer and Roe	(1934)
17.1	–	–	3.85	479.0	–	–	58.6	7.52	Shimers	(1937)
12.0	24.0	15.3	–	–	–	–	–	–	Heller et al	(1934)
12.0	25.1	–	–	–	–	–	–	–	Correll, Hughes (Sturkie)	(1946)
–	–	–	–	–	–	–	51.2	–	Altman	(1961)
–	–	–	–	–	–	–	54.2	7.39	Helbacka	(1963)

Total plasma Ca	Ultrafiltrable Ca (Hen layers)		References	
mg/100 ml	mg/100 ml	meq/l		
21.5 – 28.1	8.7 – 13.2	4.4 – 6.6	Winget and Smith	(1958)
23.9	9.45	4.73	Taylor and Hertelendy	(1961)

Plasma sodium	Plasma potassium (Hen layers)	References	
meq/l	meq/l		
138.6	3.6	Kravis and Kare	(1960)
148.0	6.4	Pudelkiewicz et al.	(1959)

Total magnesium	Ultrafiltrable magnesium (Hen layers)	References	
mg/100 ml	mg/100 ml		
3.88	2.20	Taylor and Hertelendy	(1961)
3.00 – 3.05	1.8 – 2.3	Taylor and Hertelendy	(1961)

3.3. MATERIAL AND METHODS

This experiment was carried out in the Department of Animal Physiology. The main goal of the experiment was to raise the alkali reserve of the blood and to keep the bird more or less in a state of compensated alkalosis and to watch the effect on shell quality in both hot and cold climate. A survey of the experimental design is given in table 20.

TABLE 20. Experimental design (12 layers per treatment).

Date	Treatments	Cold		Hot	
		Control	Treated	Control	Treated
July 10–14	Control period	55 °F	50–60 R.H.	85 °F	75–80 R.H.
July 17 to August 7	2% sodium acetate 3 Aqua; 140 meq Sodium per kg feed	55 °F	50–60 R.H.	85 °F	75-80 R.H.
August 8–14	Transition period No data available		65–70 °F	50–60 R.H.	
August 15 to August 28	2.1% Sodium bicarbonate; 250 meq sodium per kg	55 °F	50–60 R.H.	85 °F	75–80 R.H.

Experimental flock: The same layers were used as in the first experiment, after keeping them 10 days on the normal ration and normal temperature 65–70°F 50–60 R.H. to give the birds an opportunity to recover from the severe stress.

Birds were redistributed in a way to level out residual effects from the first experiment.

Procedure: The same methods of shell quality determination from the first experiment were used in this one with some omissions. The shell quality determinations were:

Egg weight, specific gravity, shell weight, shell percentage and shell thickness (in three parts of the shell).

Ration: The same basal ration as mentioned in table 1 was used. The alkalic part of the ration was calculated as Ca^{++}, Mg^{++}, K^{+} and Na^{+}. The acid part also was calculated as Cl', SO_4'' and PO_4'''. This calculation of acid and alkali was done in all the ingredients of the ration on the basis of feed tables.

Organic sodium compounds were used in order to raise the alkali reserve of the birds. Organic sodium was used to avoid any possibility of acidity because most of the base (sodium) will remain longer in the blood and the organic part will be secreted by the lungs after conversion into $CO_2 + H_2O$.

In the first part of the experiment, 2% sodium acetate 3 aqua was supplemented to the mash after premixing. The result of this addition was 140 mg equivalent sodium per kg. In the second part of the experiment, 2.1% sodium bicarbonate was added to the ration after premixing, bringing 250 mg equivalent sodium per kg in the ration.

Chemical determinations in the blood plasma:

The same technique was used as in the first experiment for collecting the blood. The time of taking the blood samples was equally between 1 p.m. and 3 p.m. only for hens which had laid an egg. Larger quantities of blood were used (4 cc). The following chemical determinations were carried out:

1. *Calcium and calcium + magnesium:*
   The determination was done by complexonmetric method using the VITATRON apparatus; readings were in meq /l.

2. *Chlorine:*
   The potentiometric method was used with the VITATRON apparatus.
3. *Sodium and potassium:*
   Flame Photometric determinations were used.
4. *Phosphate:*
   Calorimetric methods were used after GORTER and DE GRAAFF (1947[1]).
5. *Alkali reserve:*
   Manometric methods after PETERS and VAN SLIJKE (1932) were used.

3.4. RESULTS AND DISCUSSION

Table 21 shows the effect of the different sodium supplementations to the feed and its effect on the shell quality of birds kept in hot and cold climates.

As far as egg weight is concerned, it was noticed that egg weight was highly

TABLE 21. Shows the effect of sodium additions on birds in hot and cold climate and shell quality determinations.

		Cold environment		Hot environment		L.S.D. between[3] single groups		F. Values		
		Control	Treatment	Control	Treatment	P < 0.05	P < 0.01	Treatment	Temperature	Interactions
Shell weight (g)	Control	62.58	61.36	56.03	57.81	2.59	3.41	0.35	17.60**	0.57
	Sodium[1] A.	61.92	62.35	56.10	56.97	2.10	2.77	0.37	27.23**	0.04
	Sodium[2] B.	61.71	62.40	56.91	56.47	2.34	3.07	0.01	20.23**	0.22
Egg weight (g)	Control	5.17	5.44	4.66	4.82	3.73	4.90	1.24	8.91**	0.07
	Sodium A.	5.05	5.37	4.58	4.48	3.70	4.86	0.33	12.96**	1.27
	Sodium B.	5.02	5.16	4.54	4.63	3.88	5.10	0.34	6.43**	0.01
Shell %	Control	8.2	8.6	8.3	8.4	0.77	1.01	1.34	0.79	0.96
	Sodium A.	8.2	8.6	8.2	7.9	0.96	1.26	0.07	2.01	2.31
	Sodium B.	8.1	8.2	8.0	8.2	1.00	1.31	0.49	0.07	0.04
Specific gravity	Control	1.0787	1.0811	1.0793	1.0809	0.026	0.034	2.32	0.03	0.13
	Sodium A.	1.0780	1.0808	1.0786	1.0759	0.034	0.045	0.00	1.59	2.51
	Sodium B.	1.0780	1.0781	1.0770	1.0773	0.035	0.046	0.01	0.22	0.00
Mean shell thickness of three parts	Control	33.7	34.7	31.8	33.4	1.67	2.20	2.27	3.23(*)	0.12
	Sodium A.	32.5	34.2	31.7	31.3	1.89	2.48	0.42	3.78(*)	1.17
	Sodium B.	32.0	32.9	30.8	32.0	1.88	2.47	1.45	3.42(*)	0.42

[1] 2% Sodium acetate 3 aqua = 140 meq sodium per kg.
[2] 2.1% Sodium bicarbonate = 250 meq sodium per kg.
[3] L.S.D. = Least significant differences.
(*) Nearly significant at 10% level.
* Significant at 5% level.
** Highly significant at 1% level.

TABLE 22. Shows the effect of sodium additions on birds in hot and cold climate and plasma blood constituents.

		Cold environment		Hot environment		F. Value		
		Control	Treat-ment	Control	Treat-ment	Treat-ment	Tempe-rature	Inter-action
Magnesium meq/l	Control	4.15	4.50	3.83	3.57	0.00	8.56**	2.12
	Sodium[1] A.	4.33	4.22	4.08	3.70	0.30	5.38*	1.41
	Sodium[2] B.	4.36	4.47	3.92	3.83	0.05	4.17(*)	0.09
Calcium meq/l	Control	13.04	13.34	11.36	10.15	1.12	18.14**	1.37
	Sodium A.	13.22	13.47	11.38	10.70	0.12	29.01**	1.23
	Sodium B.	13.66	13.43	10.24	10.85	0.13	30.27**	0.57
Chlorine meq/l	Control	114.47	117.65	114.50	114.77	1.33	0.86	1.20
	Sodium A.	114.58	114.52	113.21	116.24	2.14	0.01	2.95
	Sodium B.	115.15	116.08	117.11	114.70	0.19	0.00	1.17
Potassium meq/l	Control	3.93	4.10	4.17	3.98	0.04	0.72	4.39*
	Sodium A.	3.60	3.57	3.77	3.64	0.24	0.67	0.12
	Sodium B.	3.64	3.42	3.83	3.56	3.06(*)	0.94	0.54
Sodium meq/l	Control	151.2	152.2	150.6	150.3	0.06	1.63	0.53
	Sodium A.	147.4	146.8	141.1	141.1	0.01	5.54*	0.02
	Sodium B.	145.9	143.7	141.8	145.9	0.14	0.04	2.29
Phosphate %	Control	4.65	4.85	3.65	3.25	0.18	6.61*	0.40
	Sodium A.	4.45	4.45	3.15	2.85	0.45	34.40**	0.70
	Sodium B.	4.40	4.20	2.60	2.80	0.38	38.99**	0.38
Alkali Reserve Vol. %	Control	63	63	57	59	0.25	5.19*	0.37
	Sodium A.	60	62	55	61	12.41**	11.16**	1.27
	Sodium B.	59	64	54	62	28.21**	5.33*	6.54*

[1] Sodium acetate
[2] Sodium bicarbonate
(*) Nearly significant at 10% level.
* Significant at 5% level.
** Highly significant at 1% level.

significantly lowered by heat stress. Sodium additions in the different concentrations of 140 meq and 250 meq did not show any effect.

Concerning the shell quality, there was a highly significant reduction in shell weight and a nearly significant reduction in shell thickness in the hot climate. No significant improvement was observed in shell quality as determined by shell weight, shell percentage, specific gravity and mean shell thickness of three areas, by the two sodium additions in both cold and hot environment.

As far as blood constituents are concerned, table 22 shows the effect of tem-

perature and of different concentrations of sodium supplementation to the feed on the blood constituents. It is clear from the table that calcium, calcium plus magnesium, phosphate % and alkali reserve were significantly reduced by heat stress. Chlorine and potassium were not affected by heat stress, whereas sodium was lowered significantly only during the addition of sodium acetate.

It was observed that there was a highly significant increase in alkali reserve in both cold and hot environment with addition of organic sodium compounds to the feed. This increase was more pronounced in the case of sodium bicarbonate 250 meq than in the case of sodium acetate 140 meq sodium. A significant interaction during the period of sodium bicarbonate addition indicates that increase of alkali reserve by sodium bicarbonate addition is more pronounced in the hot than in the cold environment.

HALL and HELBACKA (1959) reported that during feeding of NH_4Cl, shell thickness dropped, along with a drop in blood pH. This would indicate that the laying hens were suffering from acidosis which may account for the thin shells. HUNT and AITKEN (1962) confirmed this report and suggested that plasma bicarbonate was depressed during feeding of NH_4Cl causing thinner shells.

From the review it was observed that when the birds were under acidosis caused by feeding NH_4Cl or high concentration of CO_2, a drop in pH and a lower shell thickness were obtained. So it seems that the bicarbonate ions which are carried by the blood are reduced and also the enzyme carbonic anhydrase is inactivated by acidosis. From these observations we can suggest that if carbonic anhydrase can be activated, the calcium deposition of the egg will be better.

To activate this process, alkalosis was induced in the birds. An indication that alkalosis was obtained is given by a raise in the plasma CO_2 capacity. From these data it was indicated that though the alkali reserve was significantly raised by the different sodium additions, no significant improvement was observed in shell quality. However, it may be possible that our observation on shell quality was not representative, since the birds used in this experiment were yearlings and had been used in the preliminary experiment and consequently were exhausted. Another important point was that unfortunately the experiment was not continued for a longer time. So the number of eggs was rather small, especially from the birds under stress, whose production was very low. For these reasons the next experiment was carried on with a larger number of birds and over a longer time.

CHAPTER 4

FEED SUPPLEMENTATION AND SHELL QUALITY

4.1. Introduction

It would be of great importance to have the disposal of methods, suitable to improve shell quality, by means of feed supplementation at moderate warm temperatures. These improvements might possibly be found by adding vitamin C to the feed which was suggested to improve shell thickness through promotion of the transfer of serum calcium to the shell gland of the oviduct.

Another improvement might perhaps be made by keeping the bird in a state of moderate compensated alkalosis, in order to activate the calcium deposition in the uterus through the activation of the carbonic anhydrase, or to shift the equilibrium of the reaction activated by the enzyme to the side of improving calcium deposition. A third improvement might occur by Antibiotic additions which may improve shell thickness.

From these assumptions this experiment was carried on to show to what extent shell quality might be improved in moderate warm temperature.

4.2. Review of literature

Antibiotics and shell quality:

The effect of some of the antibiotics on egg weight and shell thickness has been studied rather recently. Gabuten and Shaffner (1952, 1954) noted that procaine penicillin at the levels of 15 and 30 ppm of diet had improved shell thickness at the end of the third week of feeding.

The data were obtained during the summer, with specific gravity of the eggs as the measure of their shell thickness.

The latter data seemed to indicate that supplementing the diet of laying chickens with procaine penicillin might result in an improved thickness of their egg shells during periods of high air temperatures, such as occur during the summer months in parts of Southern Arizona.

Bogdoroff and Shaffner (1954) reported that when penicillin was fed to the layers, an improvement was obtained in shell quality of eggs from yearling hens during July and August as measured by specific gravity. No improvement resulted when penicillin was fed to pullets during September to November. They also reported that penicillin (30 ppm procaine penicillin), was effective in maintaining or increasing the specific gravity of the eggs produced, shell breaking strength was also improved as was the calcium level in the blood serum.

Eoff et al (1962) noted that supplementing the basal diet with 50 gms/ton chlortetracycline (CTC + 0.4% Terepthalic acid TPA) reduced the incidence of checks and cracks in the shell from 4.0% to 1.6%.

On the other hand, some workers came to contrary results. HEYWANG and KEMMERER (1955) reported from their work on feeding diets supplemented with 30 ppm procaine penicillin G and birds under high temperatures of 104 degrees F, that there was no appreciable change in egg weight or shell thickness due to this supplementation.

PETERSEN et al (1958) came to the same conclusion that feeding of procaine penicillin, either before or after the introduction of high environmental temperature did not improve shell quality as determined by specific gravity of eggs.

Vitamin C and shell quality in moderate and high temperature:

THORNTON and MORENG (1958) reported that ascorbic acid at levels of 5, 10 and 20 mg and sodium ascorbate at a level of 20 mg per pound of diet had a favorable influence on shell thickness and shell strength under both 'moderate' and 'high' environmental temperature. In another study, THORNTON and MORENG (1959) found that shell thickness and shell strength were improved during 'cool' and 'warm' ambient temperatures when the diet was supplemented with 10 mg ascorbic acid per pound of diet. THORNTON (1961) showed that hens were subjected to heat stress the blood values for ascorbic acid were markedly reduced, indicating that less vitamin C was synthesized.

On the other hand HEYWANG and KEMMERER (1955) reported that supplementing an all-mash laying diet with 454 mg per pound of ascorbic acid did not improve shell quality, as measured by the ratio of dried shell weight to whole egg weight. The data were obtained during two summers under uncontrolled ambient temperatures with average maxima of 101 and 104 and average minima of 90 and 91 degrees F. HEYWANG et al (1964) also reported that the ascorbic acid supplement in a concentration of 10, 20 and 454 mg/lb had no appreciable effect on egg weight, shell thickness, or ratio of dried shell weight to whole egg weight. Results were similar at all three levels of ascorbate.

4.3. MATERIAL AND METHODS

This experiment was carried on at the Institute of Agricultural Research of Biochemical Products (I.L.O.B.).[1] The main aim of this experiment was to investigate on a larger scale whether there was any effect on shell quality by treating birds with vitamin C or sodium bicarbonate and antibiotics[2] supplementation to the feed in moderate temperature and humidity. Moreover a comparison of the effect of cool, medium and high temperatures on shell quality was made.

Experimental animals: Nearly 600 yearling hens (White Plymouth Rock), 10 months in lay, were used in this experiment for 63 days from the 18th of October till the 19th December 1964. A survey of the experimental design is given in table 23.

[1] I.L.O.B.: Instituut voor Landbouwkundig Onderzoek voor Biochemische Produkten.

[2] The birds available for this experiment were needed also in experiments with antibiotics, the design of the experiment was made in a way that possible effects of antibiotics and of interactions of the feed supplementations on shell quality could be estimated.

TABLE 23. Experimental design.

No.	Temperature	Humidity	No. hens	Feed supplementation
1	75–77 °F	50–60 R.H.	48	Basal
2	75–77 °F	50–60 R.H.	48	Basal+2.1 sodium bicarbonate
3	75–77 °F	50–60 R.H.	48	Basal+2.1 sodium bicarbonate+ 100 mg vitamin C/kg
4	75–77 °F	50–60 R.H.	45	Basal
5	75–77 °F	50–60 R.H.	51	Basal+100 mg vitamin C/kg
6	75–77 °F	50–60 R.H.	53	Basal+7 ppm proc. penicil.
7	75–77 °F	50–60 R.H.	51	Basal+100 mg vitamin C/kg+7 ppm proc. penicil.
8	75–77 °F	50–60 R.H.	51	Basal+3 ppm Ilma
9	75–77 °F	50–60 R.H.	54	Basal+100 mg vitamin C/kg+3 ppm Ilma
10	75–77 °F	50–60 R.H.	53	Basal+10 ppm Ilma
11	75–77 °F	50–60 R.H.	52	Basal+100 mg vitamin C/kg+10 ppm Ilma
12	55 °F	50–60 R.H.	24	Basal
13	85 °F	75–80 R.H.	24	Basal
1	75–77 °F	50–60 R.H.	48	Basal

Housing: Birds in the moderate warm climate were kept in laying batteries in a very large chamber. The batteries were composed of three floors of cages. Cages were equipped with automatic waterers, feed was added once per day; birds were fed *ad libitum*.

The system of heating was by means of hot water; heat was transferred to the circulating air and distributed in the whole room by means of ventilators and tubes; the system was thermostatically controlled, temperature was ranging between 75–77°F. Humidity was controlled by regulating the ventilation speed and also by spraying water on the walls and ground, the relative humidity percentage was ranging from 50–60%.

Both temperature and humidity were recorded continuously by means of a thermohygrograph.

Birds were receiving 16 hours of light by fluorescent lamps fitted in pairs all over the room and 8 hours of darkness daily.

The 'cold' and 'hot' groups were housed in the same cages as mentioned in the first experiment with the same means for control of temperature and humidity as described before.

Ration: The normal basal ration mentioned in table 2 was used, feed was prepared each two weeks. This ration was supplemented with different additives as follows:

1. *Vitamin C:* Vitamin C of HOFFMAN-LA ROCHE. The product was used in a concentration of 100 mg/kg of feed. Also combinations of added vitamin C with the other supplementations (2, 3, 4) were tried.
2. *Sodium bicarbonate:* Normal $NaHCO_3$ was used as a feed supplementation

to the main ration in a level of 2.1 % providing 250 meq of sodium per kg of ration. Sodium bicarbonate was added both separately and combined with vitamin C.

3. *Procaine penicillin:* procaine penicillin of a very high stability known as VEVOPEN 70, one of the ROYAL NETHERLANDS FERMENTATION INDUSTRIES LTD., DELFT,[1] HOLLAND Products, 1 gram of VEVOPEN 70 = 700 mg Procaine Penicillin = 700.000 International Units. It was supplemented at a concentration of 7 parts per million (= 4,9 mg procaine penicillin per kg). Procaine penicillin was tried both separately and combined with vitamin C.
4. *Ilma:* This is a new experimental antibiotic known as Lathumycine *Streptomyces Lathumensis* one of the products of the industry mentioned above. It was supplemented in a concentration of 3 and 7 parts per million of antibiotics. It was used both separately and together with vitamin C.

Chemical determinations in blood plasma:

Blood samples were obtained from the birds in the way mentioned before, and analysed for calcium, chlorine and alkali reserve. The same technique as in the second experiment was used here.

Shell quality determination:

Egg weight, specific gravity, shell weight, shell thickness and shell percentage were determined as mentioned before.

4.4. RESULTS AND DISCUSSION

1. *Sodium supplementation:*

Tables 24 and 25 show the effect of different feed supplements separated and combined and their effect on egg weight shape index and shell quality by general means and by analysis of variance.

In the case of sodium supplementation, an improvement was observed in egg weight with a significant effect compared with groups receiving the basal ration. The same effect was in the shape index with a highly significant effect. Concerning shell quality, a highly significant effect was observed in shell weight and a nearly significant effect was observed in shell thickness, while specific gravity and shell percentage did not show a significant improvement.

In the case of combining sodium plus vitamin C supplementation, the improvement in egg weight and shell quality was more pronounced. A highly significant improvement of egg weight and a significant increase in shape index were observed. In shell quality determination a highly significant increase of shell weight and shell thickness were noticed, and a nearly significant increase in shell percentage, while specific gravity was not effected. Table 26 shows the effect of sodium supplementation separately and combined with vitamin C on alkali reserve in volume percentage and on calcium and chlorine in meq/l.

[1] Koninklijke Gist- en Spiritus Fabriek N.V., Delft.

TABLE 24. Shows the effect of different feed supplements separately and combined and their effect on egg weight and shell quality in medium temperature as well as a comparison between cold, medium and hot temperature (data are represented in means).

Treatments	Egg weight g	Specific gravity	Shape index	Shell weight g	Mean Shell thickness	Shell percentage *(aercsin)* $\sqrt{X}$
Basal ration control	63.47	1.0837	72.08	553	34.83	300
B+sodium suppl.	64.61	1.0826	72.83	569	35.66	301
Basal ration control	63.47	1.0837	72.08	553	34.83	300
B+sodium+vitamin C suppl.	65.82	1.0838	72.66	588	36.16	303
B+sodium suppl.	64.61	1.0826	72.83	569	35.66	301
B+sodium+vitamin C suppl.	65.82	1.0838	72.66	588	36.16	303
Basal ration control	63.47	1.0837	72.08	553	34.83	300
Basal+vitamin C suppl.	65.20	1.0830	72.16	576	35.66	302
Basal ration control	63.47	1.0837	72.08	553	34.83	300
B+P.P.7 ppm	63.74	1.0818	72.33	556	34.66	299
B+Ilma 3	64.31	1.0823	72.83	559	34.83	299
B+Ilma 10	64.29	1.0825	72.00	567	35.00	302
P.P.7+Ilma 3+Ilma 10 suppl.	64.11	1.0822	72.38	561	34.83	300
(P.P.7+V.C)+(Ilma 3+V.C)+(Ilma 10+V.C)	64.89	1.0827	72.50	573	35.77	301
B control+P.P.7+Ilma 3+Ilma 10 suppl.	63.86	1.0828	72.26	558	34.83	300
V.C+(P.P.7+V.C)+(Ilma 3+V.C)+ (Ilma 10+V.C)	64.97	1.0828	72.41	574	35.75	301
Cold temperature	65.59	1.0834	72.50	580	36.00	302
Basal ration Medium temperature	63.47	1.0837	72.08	553	34.83	300
Hot temperature	59.06	1.0735	72.83	452	30.33	279

TABLE 25. Shows the effect of different feed supplements in different combinations and their effect on egg weight, shell quality and egg production in medium temperatures according to the analysis of variance.

Treatments	d.f.	F. Value						d.f.	F. Value
		Egg weight	Specific gravity	Shape index	Shell weight	Shell thick.	Shell %		Egg production%
Basal ration against sodium supplementation	1–16	7.83*	0.30	9.59**	20.26**	4.03(*)	0.17	1–44	2.68
Basal ration against sodium + Vit. C suppl.	1–16	28.26**	0.00	5.12*	49.35**	9.10**	3.99(*)	1–45	0.90
Sodium suppl. against sodium + Vit. C suppl.	1–10	7.14*	3.78(*)	0.37	6.22*	0.46	3.90(*)	1–91	0.27
Basal ration against Vit. C suppl.	1–16	18.56**	0.11	0.11	20.89**	4.03(*)	0.71	1–45	1.27
Basal ration against 7 P.P. + Ilma 3 + Ilma 10	3–26	3.00*	0.58	4.73**	10.42**	0.49	1.49	3–245	0.22
7. P.P. + Ilma 3 + Ilma 10 against (7.P.P.+V.C) + (Ilma 3+V.C) + (Ilma 10+V.C)	1–34	10.11**	1.63	0.34	11.36**	11.55**	2.58	1–296	3.12(*)
Basal+7.P.P.+Ilma 3+Ilma 10 against Vit. C+(7.P.P.+V.C.)+ (Ilma 3+V.C)+(Ilma 10+Vit. C)	1–52	26.77**	0.00	0.95	29.47**	16.80**	3.13(*)	1–443	1.73

** Highly significant at 1%.
* Significant 5%.
(*) Nearly significant 10%.

TABLE 26. Shows the effect of sodium bicarbonate and sodium bicarbonate with vitamin C supplementations on some blood plasma constituents. Differences are tested with analysis of variance and difference of the Student-Neuman-Keuls-test with upper limits 5% and 1%.

Alkali Reserve: vol. %	d.f. 2–30	F. Value 7.93**			
		Differences			
	Mean	Sodium+Vit. C	Sodium	UP5	UP1
Sodium+Vit. C	64.90				
Sodium	63.80	1.10		2.71	3.65
Basal	59.88	5.02**	3.92*	3.27	4.17
Calcium meq/l:					
	d.f. 2–30	F. Value 0.35			
		Differences			
	Mean	Sodium	Sodium+Vit. C		
Sodium	12.51				
Sodium+Vit. C	12.39	0.12	0.00	1.07	1.44
Basal	12.08	0.43	0.31	1.29	1.65
Chlorine meq/l:					
	d.f. 2–30	F. Value 0.25			
		Differences			
	Mean	Sodium	Sodium+V.C.		
Sodium	12.167				
Basal	12.042	0.125		6.27	8.44
Sodium+Vit. C	11.950	0.217	0.92	7.57	9.66

** Highly significant 1%.
* Significant 5%.

It was clearly observed by analysing the variance that there was a highly significant difference between the sodium additions and the basal ration as far as the alkali reserve in the blood plasma is concerned.

In order to know the real effect between the different additions the STUDENT-NEUMAN-KEULS-test was run.

From the same table it was observed that the sodium bicarbonate addition caused a significant raise in alkali reserve, but when sodium was combined with vitamin C against the basal ration, a highly significant difference was observed. In calcium and chlorine determinations no significant effect of sodium bicarbonate or vitamin C was observed in these groups.

From those results we can conclude that the sodium bicarbonate additons to the feed, caused an improvement in shell quality, egg weight and shape index and also a raise in alkali reserve. In the case that vitamin C was combined with the sodium, the effects were more prounced.

HALL and HELBACKA (1959) reported that during feeding of NH_4Cl shell thickness dropped along with a drop in blood pH. This would indicate that the laying hens were suffering from acidosis which may account for the thin shells.

HUNT and AITKEN (1962) confirmed this report and suggested that plasma bicarbonate was depressed during feeding of NH_4Cl causing thinner shells; also HELBACKA et al (1963) reported that changing the acid base balance via the

respiratory system would have the same effect as feeding NH_4Cl. Finally he came to the conclusion that exposure of laying hens to environment of high CO_2 caused a drop in blood pH and a reduction in shell thickness.

Contrary to all those reports, our work was carried on in a way to raise the blood pH or to bring the bird in a state of alkalosis instead of acidosis and to improve shell quality instead of having a weak shell. These results agree with our findings in chapter 3 which showed a high alkali reserve when the birds received sodium additions to the feed.

2. *Vitamin C supplementation:*

From table 24 and 25 we can observe the effect of different supplementations separated and combined on the egg weight, shape index and shell quality by general means and by analysing the variance. In the case of vitamin C supplementation an improvement was noticed in egg weight with a highly significant effect compared with the control groups. As far as shell quality is concerned, a highly significant improvement in shell weight and a nearly significant improvement in shell thickness were obtained while specific gravity, shell percentage and shape index did not show any significant improvement.

As was mentioned before, the combining of sodium with vitamin C showed a pronounced effect on egg weight and shell quality. In the case that sodium supplementation was taken as a basal ration, compared with the groups treated with sodium plus vitamin C in order to show the real effect of vitamin C, a significant improvement of egg weight and shell weight and a nearly significant improvement of specific gravity and shell percentage by vitamin C addition was observed.

In the case of combining all the antibiotics, feed supplementation as a control against the same combined antibiotics plus vitamin C, a highly significant improvement in egg weight, shell weight and shell thickness was observed.

In combining the basal treatments plus all the antibiotic additions against the vitamin C addition plus all antibiotics, combined with vitamin C, a highly significant improvement in egg weight, shell weight and shell thickness gain was observed and a nearly significant improvement in shell percentage.

THORNTON and MORENG (1958–1959) reported that ascorbic acid supplementation had a favorable influence on shell thickness and shell strength under both 'moderate' and 'high' environmental temperature, and also during 'cool' and 'warm' ambient temperatures. From our work in chapter 2 it was also significantly proved that vitamin C improved shell quality in hot environments. Our results are in agreement with the work of THORNTON and MORENG (1958–1959) as far as the favorable effect of vitamin C on shell quality in moderate temperature is concerned. However, specific gravity of the egg fails to show this improvement of shell quality, though it is seen in shell thickness and to some extent in shell percentage too.

3. *Antibiotic supplementation:*

From tables 24 and 25 we can see the effect of antibiotic supplementation as

procaine penicillin and Ilma separately or combined on egg weight, shape index and shell quality. From the tables we can notice that there was a significant increase in egg weight, a highly significant increase in shape index and shell weight in case of combining all the antibiotics supplementations against the basal rations.

In order to observe the real effect of each antibiotic separately, the STUDENT-NEUMAN-KEULS-test was carried on to show the real effect of each antibiotic on the different items tested for egg weight, and shell quality. The results are shown in table 27. As far as egg weight is concerned, there was a significant effect by supplementing Ilma 3 and Ilma 10 as compared with the basal ration. Specific gravity did not show any improvement. In the case of shell weight, the supplementation of Ilma 10 showed a highly significant improvement compared with Ilma 3 and procaine penicillin 7 and the basal ration, while Ilma 3 supplementa-

TABLE 27. The effect of different antibiotics feed supplements on egg weight and shell quality in medium temperature. Differences are tested with analysis of variance and difference tables of the STUDENT-NEUMAN-KEULS-test with upper limits 5 % and 1 %.

Egg weight:	d.f. 3 26		F. Value 3.00*			
	Mean	Ilma 3	Ilma 10	P.P.7	UP5	UP1
Ilma 3	64.31					
Ilma 10	64.29	2	0	0	56	75
P.P.7	63.74	57	55	0	68	85
Basal	63.47	84*	82*	27	74	92
Specific gravity:	d.f. 3 26		F. Value 0.58			
	Mean	Basal	Ilma 10	Ilma 3		
Basal	1.0837					
Ilma 10	1.0825	12	0	0	27	36
Ilma 3	1.0823	14	2	0	32	41
P.P.7	1.0818	19	7	5	35	44
Shell weight:	d.f. 3 26		F. Value	10.42**		
	Mean	Ilma 10	Ilma 3	P.P.7		
Ilma 10	5.67					
Ilma 3	5.59	8*	0	0	4	6
P.P.7	5.56	11**	3	0	5	7
Basal	5.53	14**	6*	3	6	7
Shell thickness:	d.f. 3 26		F. Value	0.49		
	Mean	Ilma 10	Ilma 3	Basal		
Ilma 10	35.00					
Ilma 3	34.83	17	0	0	39	51
Basal	34.83	17	0	0	46	58
P.P.7	34.66	34	17	17	51	63
Shell percentage:	d.f. 3 26		F. Value	1.49		
(arcsin) $\sqrt{X}$	Mean	Ilma 10	Basal	P.P.7		
Ilma 10	302					
Basal	300	2*	0	0	2	3
P.P.7	299	3**	1	0	2	3
Ilma 3	299	3**	1	0	3	3

* Significant 5 % level.
** Highly significant 1 % level.

tion showed a significant improvement compared with the basal ration. Shell thickness did not show any significant improvement with the different supplementations. In the case of shell percentage the supplementation of Ilma 10 showed a significant improvement against the basal ration and a highly significant improvement against procaine penicillin 7 and Ilma 3.

A lot of workers discussed the problem of antibiotic feed supplementation and shell quality. GABUTEN and SHAFFNER (1952–1954), BOGDOROFF and SHAFFNER (1954) and EOFF et al (1962) all came to the conclusion that antibiotic feed supplementation in forms of procaine penicillin and chlortetracycline improved shell quality in the summer months of the year. This agrees with our findings concerning shell weight and shell percentage. Though in our case penicillin did not show any improvement of egg weight or shell quality, Ilma 10 improved egg weight, shell weight and shell percentage, wherever Ilma 3 improved egg weight and shell weight only.

From the last data it was quite clear that specific gravity in general did not show any improvement, and referring to our work in chapter 2, it was quite clear that here too specific gravity was not a good measure for improvement of shell quality.

The significant rise in shape index noticed in the data may be a reflex from the significant rise in egg weight. Increased shape index with increased egg weight might rather be an indication for an improved water balance.

Since shell weight is highly correlated with egg weight, so the significances observed in shell weight are a reflex of the raise in egg weight. It was also noticed that egg production did not show any improvement by the three supplementations, table 25. However, egg production can not be a good indication in the case of this experiment since the production was taken in a short period and the hens were in the last season of production.

4. *Effect of cold, medium and high temperature on egg weight and shell quality:*

Table 28 shows the effects of hot, medium and cold temperatures on egg weight, and shell quality. Differences are tested with analysis of variance and difference-tables of the STUDENT-NEUMAN-KEULS-test. Concerning egg weight, there were highly significant differences between eggs of cold, medium and hot treatments. By the STUDENT-NEUMAN-KEULS-test analysis it was observed that eggs obtained from medium temperature were smaller than those of cold, and those of hot environment were smaller than those of medium temperatures, although the difference between hot and medium temperature was twice as much as that between medium and cold temperature. F values of specific gravity showed that there was a highly significant difference between temperatures according to the STUDENT-NEUMAN-KEULS-test.

Cold and medium temperatures showed highly significantly better specific gravity than the hot environment, but there was no difference between medium and cold temperature.

As far as shell quality is concerned, shell weight, shell thickness and shell percentage all showed highly significant temperature effects. According to the

TABLE 28. Shows the effect of hot, medium and cold temperatures on egg weight and shell quality. Differences are tested with analysis of variance and difference, tables of the STUDENT-NEUMAN-KEULS test with upper limits 5% and 1%.

Egg weight:	d.f. 2 21	F. Value 23.78**			
	Mean	Cold	Medium	UP5	UP1
Cold	65.59				
Medium	63.47	2.12**	0.00	1.39	1.86
Hot	59.06	6.53**	4.41**	1.67	2.12
Specific gravity:	d.f. 2 21	F. Value 13.45**			
	Mean	Medium	Cold		
Medium	1.0837				
Cold	1.0834	0.0003	0.0000	0.0034	0.0046
Hot	1.0735	0.0102**	0.0099**	0.0041	0.0052
Shell weight:	d.f. 2 21	F. Value 43.34**			
	Mean	Cold	Medium		
Cold	5.80				
Medium	5.53	2.7*	0	21	28
Hot	4.52	1.28**	1.01**	25	32
Shell thickness:	d.f. 2 21	F. Value 32.06**			
	Mean	Cold	Medium		
Cold	36.00				
Medium	34.83	1.17*	0	1.09	1.45
Hot	30.33	5.67**	4.50**	1.31	1.66
Shell percentage:	d.f. 2 21	F. Value 33.81**			
(arcsin) $\sqrt{X}$	Mean	Cold	Medium		
Cold	302				
Medium	300	2	0	5	6
Hot	279	23**	21**	5	7

** Highly significant 1% level.
* Significant 5% level.
(*) Nearly significant 10% level.

STUDENT-NEUMAN-KEULS-test, hot temperature affected shell quality highly significantly more than medium temperature and the medium temperature was also harmful to the shell as far as shell weight and shell thickness are concerned, as compared with the cold environment. This work agrees to a great extent with our previous experiment discussed in chapter 2 with the addition that medium warm temperatures too, have an unfavorable effect on egg weight and shell quality.

CHAPTER 5

MICROSTRUCTURE OF THE SHELL

5.1. INTRODUCTION

In view of the influence of heat stress of the birds on externally measured shell quality, it was of interest to study the influence of this heat stress on the thickness and microstructure of the different layers of the egg shell.

The shell is generally considered to be made up of the following parts:

The inner and the outer shell membranes, the mamillary layer, the spongy layer and the cuticle. Several pore canals are piercing these layers.

The shell is gracefully curved to fit the taut egg membranes, over which the secretions of the uterus pour out and harden, much like cement. The domed architecture of the shell contributes to its strength, it utilizes the principle of the arched stone bridge, designed to bear extraordinary weight on its convex surface. Additional strength is given to the shell by the radial orientation of crystals in its outer surface. If the crystals were parallel to the surface the shell might scale and thus be weakened.

5.2. REVIEW OF LITERATURE

Although there have been a number of studies which were concerned with shell thickness, little attention has been given to the microscopic structure of the shell obtained in hot climate.

ROMANOFF and ROMANOFF (1949) reviewed most of these studies. The shell is described as having two membranes composed of keratin fibre, the outermost attached to the shell by mammillae. These knob-like structures are the beginning of the mammillary layer. It is rich in a fibrous protein matrix which ALMQUIST (1934) described as being a collagen – like substance. The spongy layer is next, and is misnamed, since it is predominantly calcite crystals bound together by a sparse matrix. STEWART (1935) reported that the crystals in the outer portion of this layer have their principal axis perpendicular to the shell surface. The cuticle which covers the shell is considered to be mainly mucin. SIMKISS and TYLER (1957) investigated the matrix histochemically and found that in the so-called spongy layer, the matrix consisted of a protein-muco-polysaccharide complex. There also appeared to be a protein core in the mammillae of the mammillary layer.

Concerning the heat stress and its effect on shell quality, MATHER et al (1962) reported from their work on the influence of environmental temperature and microscopical structure of the egg shell, that the quantity of matrix was not influenced by an elevated temperature to the same degree as the shell thickness. He also reported that there is no clear-cut relationship between structure and shell thickness. Shell-less eggs were found to have no matrix, thus indicating that this structure is essential for formation of the shell.

Recently SIMONS and WIERTZ (1963) reported a study by electronmicroscope on shells of the hen's egg. They suggested that the density of the organic matrix in the shell bears a positive relation to shell strength.

Microscopic structure of the different layers of the shell:

1. *The cuticle:* The external surface of the shell is mostly coated with an extremely thin transparent protein. In this thin layer, lay the mouths of the pores (MORAN and HALE, 1936).

Concerning the thickness of the cuticle, ROMANOFF and ROMANOFF (1949) reported that the cuticle is usually deposited on the hen's egg to a depth of 0.005 to 0.010 mm. It is present in greatest quantity in the small concave areas about the mouths of pores.

The so-called plaques (MARSHALL and CRUICKSHANK, 1938) of matrix material in the mouth of the pores, must be considered to be a part of the cuticle because they are firmly united with the inner layer of the cuticle.

2. *Pores:* A pore is an opening in the surface of the shell forming the mouths of the pore canals.

The pore canals traverse the spongy layer approximately at right-angles to the surface of the shell and form connecting passages between the exterior of the shell and the network of air spaces in the mammillary layer. Each pore canal is of smallest bore at the base of the spongy layer; from this point the canal gradually widens toward its mouth, where the diameter is largest. The mouth of the canal opens into a narrow, branched groove which lies lengthwise at the bottom of a shallow, irregularly oval indentation on the surface of the shell (ROMANOFF and ROMANOFF, 1949).

The entire pore system is filled with a matrix of protein fibers which stain in the same manner as the shell membranes. The fibers close the pore canal and its mouth and fill the groove and the oval depression to the level of the surrounding shell surface. These matrix filled areas are called 'plaques' by MARSHALL and CRUICKSHANK (1938).

3. *The spongy layer:* STEWART (1935) reported that this layer comprises approximately two-thirds of the entire shell thickness, while under even moderate magnification it shows no definite structure.

KELLY (1901) made a good crystallographic study of the spongy layer. She pointed out that it was chiefly made up of small calcite crystals very thickly interlayered. In the lower portion of it calcium phosphate crystals are more or less general.

ROMANOFF and ROMANOFF (1949) reported that the spongy layer is very compact, although numerous microscopic canals traverse its entire depth at irregular intervals. The shell looks spongy only after decalcification. Its deposition on the fragile mammillary layer confirms the shape of the egg and gives rigidity and strength to the shell.

4. *Mammillary layer:* Immediately adjacent to the membranes there is a profusion of knob-like processes. The mammillary layer rests on the outer surface

of the shell membrane and is partially embedded in it. The mammillary layer is composed of numerous roughly conical knobs or mammillae.

NATHUSIUS (1871a, 1874) made an excellent study of this layer and presented a very complete picture of it: A liberal translation follows: 'Against the inner surface (of the membranes) there is a large number of peculiarly protruding processes. They seem to be spherical, separate, and discrete from the remainder of the shell. The mammillae are separated by interspaces and these make a connective system of spaces which communicate with the inner membrane and the so-called pore canals.'

The knobs are exceptionally large spherulite crystals of calcite as was shown by KELLY (1901), CLEVISCH (1913) and SCHMIDT (1924). With the aid of the polarisation microscope, KELLY was able to identify crystals of calcium phosphate here, though the complete optical properties were unobtainable due to the small size of the crystals.

Other experiments by NATHUSIUS (1893) provided some interesting facts on the formation of this layer. This worker removed eggs from the oviduct in various stages of development and noticed that one could obtain eggs which showed a complete mammillary layer without the general chalk layer.

BLASIUS (1867) reported an estimate of the mammillary layer thickness. In the hen's egg the thickness is about 0.11 mm, or approximately one-third that of the entire shell. The height of a single mammilla corresponds to the thickness of the mammillary layer and the diameter is 0.096 to 0.144 mm.

5. *The shell membranes:* The membranes, two in number, are made up of a profuse network of fibers, the inner one (next to the egg white) being a very fine mat. At the blunt end of the egg the two layers separate, during cooling and by evaporation, to form the air cell.

A number of workers have attempted measurements of the membrane thickness (NATHUSIUS, 1868; HAYS and SUMBARDO, 1927; SMITH, 1930). The values vary considerably, because of the difficulties of obtaining representative mounted membranes. The overall thickness of the membranes probably ranges from 0.07–0.11 mm.

KELLY (1901) and CLEVISCH (1913) have definitely shown that calcium carbonate is absent in these layers and calcareous material was in evidence.

5.3. MATERIALS AND METHODS

This work was carried out in the soil Survey Institute Micropedology Department. The main target of this work was to measure and observe the microstructure of the different shell layers. Shells used in this experiment were representing all the treatments mentioned in the first experiment.

After all the shell quality determinations were carried on (washing and drying), shells were collected according to the hen number. All the 48 hens were represented in this study and 288 shells were used. From the equator three parts were taken, each part consisted of nearly 1,5 square centimeters of shell. For the preparation of the thin sections, the method of JONGERIUS and HEINTZBERGER (1963) was followed.

Description of method for preparing thin sections:

1. *Impregnation:* Three pieces of shells were impregnated in small soft metal boxes which contained a small quantity of quick hardening plastic (Vestopal-H. plastic; cyclonox for hardening + accelerator for acceleration of the hardening process). Shells were pinced in a small layer of plastic with forceps. Liquid slow hardening plastic was poured on the shells and the boxes were kept under a fairly high vacuum for 24 hours. Boxes were kept for one week in a well ventilated fume cupboard for final hardening.
2. *Sawing:* The metal boxes were torn and the plastic blocks containing the shells were sawed with a special electric saw which was cooled with a special oil.
3. *Grinding:* After sawing, the blocks were ground by hand with a declining-grain-size powder (from coarse to very fine). Oil was used for cooling; blocks were washed with petrol and polished with diamond paste on a rotating disc.
4. *Mounting:* After polishing, the blocks were rinsed with petrol, dried and then mounted for fixing on the slides with plastic. After 24 hours the polished blocks were dried and fixed and ready for sawing.
5. *Sawing the mounted sections:* With the saw used in step 2, the fixed blocks on the slides were sawed to 2 mm thickness.
6. *Thin grinding and polishing:* In this operation the section is reduced from 2 mm to a thickness of 40–20 microns by means of a special grinding machine. After reaching 40–20 microns the fine finishing of the slide is made by hand as in step 3.
7. *Covering:* After the thin section had been rinsed and dried, a glass cover slide was fixed by means of plastic. After drying, the slide was cleaned with a razor blade and acetone and labels were attached.

Finally slides were tested by a normal microscope with a micrometer eye piece to measure the different layers in $^1/_{100}$ mm.

Some slides representing the hot and cold environment were tested by the polarised light for microphotography.

5.4. Results and discussion

Table 29 shows the mean of the different shell layers in a 100th of a mm as affected by different environmental temperature and vitamin C additions. From the table we can conclude that the first period combined as a general control (control + acclimatization) for the whole data did not show any irregular fluctuations in the different four layers of the shell significantly.

1. The cuticle:

From table 29 and 30 it was clearly noticed that the high temperature (stress) was affecting the cuticle to a great extent compared with the cold temperature; according to analysis of variance and covariance, a highly significant temperature effect was present. Vitamin C additions did not show any significant improvement. From table 31 it was noticed that the thickness of the cuticle shows

TABLE 29. Means of the different shell layers as affected by different environmental temperature and vitamin C additions.

	Periods	Cold environment		Hot environment	
		Control	Treatment	Control	Treatment
Cuticle	Control and acclimat.	2.97*	3.09	2.67	2.61
	Stress	3.00	2.75	1.33	1.67
	Vitamin C additions	1.38	2.75	1.54	1.46
Spongy layer	Control and acclimat.	15.83	15.61	19.53	19.58
	Stress	16.58	16.42	14.08	14.67
	Vitamin C additions	15.96	16.96	12.95	15.79
Mammillary layer	Control and acclimat.	13.00	13.19	12.70	13.28
	Stress	12.00	12.33	10.08	10.92
	Vitamin C additions	13.21	12.55	10.50	11.63
Shell membranes	Control and acclimat.	5.92	5.19	4.80	5.25
	Stress	4.25	4.17	4.58	4.00
	Vitamin C additions	3.42	4.17	3.50	3.88

* Values are in 100th of a mm.

TABLE 30. Analysis of variance and covariance showing the effect of stress and vitamin C additions on the different layers of the shell.

Treatments	Layers of the shell	Calculated F Value			
			Treatment	Temperature	Interactions
Stress period	Cuticle	A.V	0.50	50.97**	2.29
		A.Co	–	44.31**	–
	Spongy layer	A.V	0.50	4.77*	0.15
		A.Co	–	6.92*	–
	Mammillary layer	A.V	0.97	4.27*	0.18
		A.Co	–	4.25*	–
	Shell membranes	A.V	0.54	0.03	0.30
		A.Co	–	0.06	–
	Total shell thickness	A.V	0.10	6.69*	0.18
		A.Co	–	7.54*	–
	Cuticle	A.Co	0.74	4.30*	0.74
	Spongy layer	A.Co	11.58**	18.51**	11.85**
Vitamin C additions	Mammillary Layer	A.Co	0.12	10.69**	0.02
	Shell membranes	A.Co	10.78**	0.16	10.95**
	Total shell thickness	A.Co	8.56**	19.17**	10.58**

* Significant at 5% level.
** Highly significant at 1 % level.
A.V. = Analysis of variance.
A.Co. = Analysis of covariance against control and acclimatization 2 & 3.

a highly significant correlation with the thickness of the shell membranes and with shell strength measurements. These correlations are positive with shell thickness and breaking strength and negative with deformation.

Concerning the thickness of the cuticle, ROMANOFF and ROMANOFF (1949) reported that the cuticle is usually deposited on the hen's egg to a depth of 0.005 to 0.010 mm.

It is seen from our data that the thickness of cuticle in 100th of a mm was varying from 3.00 in the cold climate to 1.33 in the hot climate. The reduction in cuticle thickness from the cold to the hot climate was statistically significant.

This reduction is probably due to the lower mucin secretions of the uterus in the case of heat stressed birds. The low estimate of cuticle thickness from shells of hens kept in cold climate as compared with the estimates of ROMANOFF and ROMANOFF, was due to the technique used in preparing the samples by washing the shells to clean them from albumin traces and drying them in the oven. So it may be that some cuticle was removed by washing and others were shrunk by drying.

From the pictures we can see that shells of stressed hens have a deteriorated cuticle as compared with shells coming from birds in cold environment.

2. The spongy layer:

From table 29 and 30 it was clear that the high temperature (stress) was affecting the spongy layer to a great extent as compared with the cold temperature. A significant temperature effect was present according to the analysis of variance and covariance. Vitamin C addition showed a highly significant improvement in that layer, mainly in the hot climate. From table 31 it is clear that the thickness of the spongy layer shows a highly significant positive correlation with total shell thickness, a significant positive correlation with breaking strength and a significant negative correlation with deformation.

As far as the thickness of the spongy layer is concerned, STEWART (1935) reported that this layer comprises approximately two thirds of the entire shell thickness. This agrees with the findings of this work that the spongy layer is nearly two thirds of the total layers. This was true in shells obtained in cold

TABLE 31. Correlation coefficients of different shell layers and shell measurements (hen averages) corrected correlations for temperature and treatment effect.

	Spongy layer	Mammillary layer	Shell membranes	Shell thickness	Deformation	Breaking strength
Cuticle	0.0842	0.2345	0.4137**	0.4848**	–0.5179**	0.4042**
Spongy layer	–	0.1417	0.0875	0.6991**	–0.3277*	0.3141*
Mammillary layer	–	–	0.1830	0.6253**	–0.3218*	0.2964*
Shell membrane	–	–	–	0.4937**	–0.4404**	0.3596*
Shell thickness	–	–	–	–	–0.5683**	0.4866**
Deformation	–	–	–	–	–	–0.7898**

* Significant at 5% level.
** Significant at 1% level.

climate but it was greatly reduced proportionally in the case of hot environment.

From these results we can conclude that the spongy layer is greatly affected by heat stress on the birds. It is probably due to the lower secretion of calcium and in general the lowering in the thickness of this layer by heat stress in causing the general reduction in shell thickness since they are highly correlated together.

KELLY (1901) made a good crystalographic study of the spongy layer. She pointed out that it was chiefly made up of small calcite crystals very thickly interlayered. From that work comparing polaroised microphotographic pictures of different shells obtained from cold and hot climate, we can conclude that shells of cold climate showed a very clear structure of crystals. The spongy layer showed very well compacted parallel columns attached together and very firmly cemented to its uneven surface. On the other hand, shells of hot climate showed that the colomunal structure is totally destroyed, the crystal columns are not running through the spongy layer but they are short, loose and irregular. A lot of air gaps are formed. Besides that, the amount of calcium structure was lowered proportionally to the constant amount of matrix as it was clear from the polarised light.

From these observations on the spongy layer and to what extent it is reduced by heat effect we can explain why the shell is weakened. Since the shell in its curvness is like an arched bridge held with parallel columns running from the spongy layer to the mammillary layer, and those columns are well attached together by means of the organic matrix, so any destruction in that system such as irregular short, loose columns and air gaps will lead to a very weak shell structure.

3. The mammillary layer:

From table 29 and 30 it was clearly observed that the high temperature was affecting the mammillary layer to a great extent as compared with the cold temperature. A significant temperature effect was present according to the analysis of variance and covariance. Vitamin C additions did not show any significant improvement. From table 31 it can be seen that the thickness of the mammillary layer shows a highly significant positive correlation with shell thickness, a significant positive correlation with breaking strength and a significant negative correlation with deformation.

Concerning the estimates of the thickness of the mammillary layer, BLASIUS (1867) reported that in the hen's egg the thickness is about 0.11 mm or approximately one third that of the entire shell.

The weight of a single mammilla corresponds to the thickness of the mammillary layer. This agrees with the findings of this work except in the case of the hot climate where the height was lowered proportionally.

NATHUSIUS (1871 a, 1874) reported that against the inner surface (of the membranes) there is a large number of peculiarly protruding processes discrete from the remainder of the shell. The mammilla are separated by interspaces and these make a connective system of spaces which communicate with the inner membrane and the so-called pore canals. Comparing the polarised microphotogra-

phic pictures obtained from the different shells, from hot and cold environment, we can conclude the following:

The mammillary layer in the cold climate is composed of numerous roughly conical knobs or mammillae which are lightly compressed, side by side, in a single stratum. The diameter of each mammilla diminishes toward the base, which terminates within the surface of the shell membrane. So the mammillary layer is a foundation to the columns of the spongy layer. The mammillary knobs are penetrated and embedded by means of different protrusions by the fibers of the shell membranes and are thus firmly attached to these membranes. This attachment together with the different matrix attachments to the columns give the elasticity to the shell and enables it to bear extraordinary weight.

On the other hand the mammillary layer in the hot climate showed a complete irregular lose construction. Besides the significant lower thickness of this layer, the columnal crystal structure running from the spongy layer to the mammillary layer is totaly destroyed. The mammillary knobs are misshaped and varying in size from large to small. The attachments of the knobs in the shell membranes are missing, but the total matrix in general is not reduced proportionally. From this view we can conclude that in case of hot weather this layer is very weakly formed and it does not give any strength to the shell.

4. Shell membranes:

From table 29 and 30 it was clearly noticed that there was no significant effect of temperature on the thickness of shell membranes. In hot and cold climates the shell membranes did not show any differences in thickness. In the case of vitamin C additions, a highly significant treatment effect was observed according to the analysis of covariance but only in the cold climate.

From table 31 it can be observed that the thickness of the shell membranes shows a highly significant positive correlation with the thickness of the cuticle and with the shell thickness, a significant positive correlation with breaking strength and a highly significant negative correlation with deformation.

A number of workers have attempted measurements of the membrane thickness (NATHUSIUS, 1868; HAYS and SUMBARDO, 1927; SMITH, 1930). The values vary considerably because of the difficulties of obtaining representative mounted membranes. The overall thickness of the membranes probably ranges from 0.07–0.11 mm. In this work, estimates ranging from 0.034–0.059 of a mm were found. This of course is rather lower than the estimates of workers which will be due to the technique mentioned before, used here by drying the shell which may have caused a certain shrinkage of the membranes.

From these data we could not find any significant effect of high temperature on the thickness of the shell membranes. This may be explained by the assumption that the secretion of albumin and calcareous materials by the oviduct is not as greatly affected by heat stress as the calcium secretions are affected, since the thickness of this layer is highly correlated with the different shell quality determinations, so it is of great importance to the total shell strength and elasticity of the shell structure.

CHAPTER 6

CONCLUSIONS

From the previous discussions we can conclude that high environmental temperature and humidity are very harmful to hen layers; it was quite clear that egg weight, shell quality and plasma blood calcium were significantly depressed.

It is suggested that low shell quality and low plasma blood calcium are due to a lower metabolism of the hen resulting from a reduced thyroxin output of the thyroid during hot environment. That suggestion can be confirmed by the work of GALPIN (1938) who reported a seasonal variation in thyroid gland weight with the low point in July (under the climatic conditions prevailing in Scotland). ASMUNDSEN and PINSKY (1935), REINEKE and TURNER (1942), GUTTERIDGE and PRATT (1946) and GUTTERIDGE and NOVIKOFF (1947) came to the conclusion that a synthetic source of thyroxin (thyroprotein) and desiccated thyroid as a feed supplement improved shell quality significantly during periods of hot weather through the activation of the metabolic rate.

From our (previous) work it was also noticed that vitamin C supplementation to the diet of (hen) layers kept in hot and medium environments improved shell quality significantly, according to the literature reviewed, metabolic rate and the ability to synthesize egg shell constituents are related. If this is true, then it seems probable that a reduced metabolic rate may also be associated with partial loss in ability by the animal organism to synthesize certain compounds which are physiologically essential for the formation of the shell.

THORNTON (1961) showed that when hens were subjected to heat stress, the blood values for ascorbic acid were markedly reduced, indicating that insufficient vitamin C was being synthesized. Since vitamin C has been shown to be involved in the utilization and metabolism of both organic and inorganic nutrients in bone, it seemed probable that its role in egg shell formation could be included in either one or both phases. So we can suggest that the real function by which vitamin C is performing appears to be essentially mediated through the thyroid gland.

Concerning the results obtained about the alkali reserve (O_2 volume percent) in the plasma in the different hot and cold environments and with different sodium supplementations, it was clear that the organic sodium compounds used, raised the alkali reserve significantly. It also means that the birds were under a state of alkalosis and that the hot environment showed a low alkali reserve as compared with a cold climate. In the case of medium temperature, the addition of the organic sodium compounds also raised the alkali reserve significantly with a significant improvement in egg weight and shell quality. This improvement was more pronounced when vitamin C was combined with sodium. The only suggestion to this phenomena is that the bicarbonate content of the blood is increased and more $CaCO_3$ may be deposited, and the enzyme carbonic anhydrase may also be activated by alkaline media in the blood.

It appears that much further work remains to be done on the acid base-balance problems in hen layers.

Concerning the significant effects on egg weight and some shell characters with antibiotic supplementation in medium temperature, it may be that antibiotics have an inhibiting effect on some intestinal bacteria which may improve the metabolic rate and calcium deposition.

It is a clear fact that layers under heat stress layed weak egg shells; this was proved by a study of the microstructure of the egg shell. It was clear that shells obtained from hens kept on high environmental temperature showed a deteriorated microstructure in the different layers significantly (cuticle, spongy layer, mammillary layer and shell membranes). Irregular columnar structure and a lot of gaps which lead to a fragile spongy structure were observed. This fact was naturally due to the lower secretion of calcium from the uterus in heat stress.

The role of vitamin C in improving some layers, like the spongy layer, significantly is a clear reflex of more calcium deposition on the shell.

CHAPTER 7

SUMMARY

The effects of cold and hot environment in a form of fluctuating and constant temperature on egg weight, shape index, formation period, clutch size and different estimates of shell quality as deformation, breaking strength, shell thickness specific gravity and shell percentage were determined.

A constant temperature of 85°F and humidity of 75–80% (heat stress) depressed egg weight, shell quality, shape index and plasma blood calcium significantly, formation period was retarded significantly, and clutch size was reduced compared with cool environment of 55°F and 50–60% relative humidity.

Fluctuating temperature of 85°F and 75–80% R.H. during 10 hours a day and 65–70°F and 50–60% R.H. during 14 hours night did not show any harmful effect on the mentioned items compared with cool environment.

Correlations based on hen averages and per egg showed significant negative correlations between deformation and breaking strength, specific gravity, mean shell thickness, shell thickness of different parts of the egg and shell percentage.

The influence of dietary ascorbic acid HOFFMAN-LA ROCHE in concentration of 50 mg per kg of ration in high temperature and 100 mg per kg of ration in medium temperature in the diet of laying hens under medium, high and cold temperatures on different shell and egg estimates wase studied.

In high temperature, egg weight, specific gravity and formation period were not affected by the presence of vitamin C, while shell quality estimates as deformation, breaking strength, mean shell thickness and shell percentage were improved significantly. Plasma blood calcium was raised significantly by vitamin C and shape index was significantly shifted towards the direction of oval shape.

In medium temperature it was clear that vitamin C as such or combined with sodium bicarbonate or with antibiotics raised egg weight and shell quality significantly. So it is a fact that ascorbic acid supplementation in the diet of a laying hen was effective for the maintenance of egg shell strength during periods of continuous heat stress.

Changing the acid base balance by sodium supplementation 2.1% sodium bicarbonate = 250 meq sodium/kg of feed and 2% sodium acetate = 140 meq sodium/kg feed in the diet of laying hens kept in cold, medium and warm temperatures resulted in raising the alkali reserve of the plasma blood significantly and a state of compensated alkalosis is produced. In medium temperature, egg weight and shell quality were significantly improved by sodium bicarbonate administration.

Antibiotics in small parts per million given combined as procaine penicillin and Ilma showed a significant improvement in egg weight, shape index and shell weight. In general, procaine penicillin alone did not show any improvement on egg weight or shell quality. Ilma in a concentration of 10 ppm showed better results than 3 ppm.

Comparing different temperatures as mentioned before (high, medium and low temperatures), it was proved significantly that a high temperature is more harmful to egg weight and shell quality than a medium temperature and that the latter is more harmful than a low temperature.

A study of the microstructure of the shell in relation to high temperatures was performed. The results showed that layers kept under heat stress produced eggs of a very weak structure. Cuticle, spongy layer and mammillary layer were deteriorated and lowered significantly in their thickness as compared with those in cool environment. The columnal crystal structure of the spongy layer deposited on the mammillary layer is totally deteriorated. The mammillary knobs are misshapen. The attachment of those knobs in the shell membranes is missing. The spongy layer shows many gaps. From the correlations it was clear that breaking strength was positively correlated with the thickness of cuticle, spongioza, mammillary layer and shell membranes.

Vitamin C additions improved significantly the total shell thickness and thickness of the spongy layer and the shell membranes.

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CHAPTER 8

SAMENVATTING EN CONCLUSIES

1. SAMENVATTING

Het effect van een koude en warme omgeving in de vorm van een fluctuerende en een constante temperatuur, op eigewicht, vormindex, vormingstijd, serielengte in de verschillende schaalkwaliteitskenmerken, zoals doorbuiging, breuksterkte, schaaldikte, soortelijk gewicht en schaalpercentage werd onderzocht.

Bij een constante temperatuur van 85°F en een relatieve vochtigheid van 75–80% (warmte-stress) werden in vergelijking met een lage temperatuur van 55°F en een relatieve vochtigheid van 50–60% het eigewicht, de schaalkwaliteit, de vormindex en het calciumgehalte van het bloedplasma significant verminderd, de tijdsduur voor eivorming werd significant verlengd en de serielengte werd verkort.

Een fluctuerende temperatuur van 85°F en 75–80% relatieve vochtigheid gedurende 10 uur per dag, afgewisseld met 65–70°F en 50–60% relatieve vochtigheid gedurende 14 uur 's nachts vertoonde vergeleken met een lage omgevingstemperatuur geen enkel nadelig effect op de genoemde kenmerken.

Correlaties gebaseerd op gemiddelden en per ei vertoonden significante negatieve waarden tussen doorbuiging en breuksterkte, soortelijk gewicht, gemiddelde schaaldikte, schaaldikte op verschillende delen van het ei en schaalpercentage.

De invloed van ascorbinezuur, HOFFMAN-LA ROCHE, in het rantsoen van leghennen in een concentratie van 50 mg/kg voer (bij hoge temperaturen) en 100 mg/kg voer (bij matig hoge temperaturen) werd onderzocht bij hoge, matig hoge en lage temperatuur op verschillende schaal- en eikenmerken.

Bij hoge temperaturen werden eigewicht, soortelijk gewicht en vormingsduur niet beïnvloed door de aanwezigheid van vitamine C, terwijl de schaalkwaliteitskenmerken, zoals doorbuiging, breuksterkte, gemiddelde schaaldikte en schaalpercentage significant verbeterd werden. Het calciumgehalte in het bloedplasma werd door vitamine C significant verhoogd en de vormindex werd significant verschoven naar de ovale vorm.

Bij matig hoge temperaturen bleek vitamine C alleen of gecombineerd met natriumbicarbonaat of met antibiotica het eigewicht en de schaal-kwaliteit significant te verbeteren.

Het is daarom een feit dat een toevoeging van ascorbinezuur aan het rantsoen van leghennen effectief is voor het behoud van de schaalsterkte gedurende perioden van aanhoudende warmte-stress.

Een verandering van het zuur-base evenwicht door toevoeging van 2,1% natriumbicarbonaat = 250 meq natrium/kg voer en 2% natriumacetaat = 140 meq natrium/kg voer in het rantsoen van leghennen, bij lage, matig hoge en hoge temperaturen resulteerde in een significante verhoging van de alkalireserve

van het bloedplasma en een toestand van gecompenseerde alkalosis werd opgewekt.

Bij matig hoge temperaturen werden het eigewicht en de schaalkwaliteit door natriumbicarbonaattoevoeging significant verbeterd.

Antibiotica toegediend in enkele delen per miljoen, gecombineerd gegeven als procainepenicilline en Ilma vertoonden een significante verbetering van het eigewicht, de vormindex en het schaalgewicht. In het algemeen vertoonde procainepenicelline alléén geen verbetering van het eigewicht of de schaalkwaliteit. Ilma vertoonde in een concentratie van 10 dpm betere resultaten dan in een concentratie van 3 dpm.

Bij vergelijking van de verschillende temperaturen zoals eerder vermeld (hoge, matig hoge en lage temperaturen) bleek dat hoge temperaturen nadeliger zijn voor het eigewicht en de schaalkwaliteit dan matig hoge temperaturen en de laatste hebben een schadelijker invloed dan de lage temperaturen.

Er werd een onderzoek verricht van de microstructuur van de schaal in verband met de hoge temperaturen. De cuticula, de spongieuze en de mamillaire laag raakten in verval en werden significant dunner dan bij lage omgevingstemperaturen.

De kolomkristallijne structuur van de spongieuze laag, afgezet op de mamillaire laag is totaal in verval. De mamillaire knobbels zijn misvormd. De hechting van deze knobbels in de schaalvliezen ontbreekt. De spongieuze laag vertoont veel holten. Uit correlatieberekeningen bleek duidelijk dat de breuksterkte positief gecorreleerd was met de dikte van de cuticula, van de mamillaire laag en van de schaalvlezen.

Vitamine C-toevoegingen verbeterden de totale schaaldikte en de dikte van de spongieuze laag en van de schaalvlezen significant.

2. Conclusies

Uit de voorgaande discussie kunnen wij concluderen dat een hoge omgevingstemperatuur en vochtigheid zeer schadelijk voor leghennen zijn. Duidelijk is gebleken dat het eigewicht, de schaalkwaliteit en het Ca-gehalte van het bloedplasma significant daalden.

Verondersteld wordt dat de geringe schaalkwaliteit en het lage Ca-gehalte in het bloedplasma te wijten zijn aan een lagere stofwisseling van de hen, een gevolg van een verminderde thyroxinesecretie van de schildklier in een zeer warm klimaat. Deze veronderstelling wordt bevestigd door Galpin (1938), die melding maakt van een seizoensvariatie van het schildkliergewicht met een minimum in juli (onder Schotse klimatologische omstandigheden).

Asmundsen en Pinsky (1935), Reineke en Turner (1942), Gutteridge en Pratt (1946) en Gutteridge en Novekoff (1947) kwamen tot de conclusie dat synthetische thyroxine (thyroproteïne) en gedroogde schildklier, aan het voeder toegevoegd, door activering van de stofwisseling de schaalkwaliteit significant verbeterden gedurende perioden van zeer warm weer.

In ons (voorgaand) onderzoek werd eveneens gesignaleerd dat een vitamine-C

toevoeging aan het rantsoen van leghennen in een zeer warm en matig warm klimaat, de schaalkwaliteit verbeterde. Volgens de besproken literatuur staan het stofwisselingsniveau en het vermogen tot synthese van eischaalbestanddelen met elkaar in verband. Als dit juist is, dan lijkt het waarschijnlijk dat een verminderde stofwisseling wellicht samengaat met een gedeeltelijk verlies in vermogen van het dierlijk organisme om bepaalde bestanddelen te synthetiseren, die fysiologisch essentieel zijn voor de schaalvorming.

THORNTON (1961) toonde aan dat wanneer hennen worden blootgesteld aan hitte-stress, de gehalten aan ascorbinezuur in het bloed belangrijk daalden, waarmee aangetoond werd dat onvoldoende vitamine-C werd gesynthetiseerd. Daar gebleken is dat vitamine-C een rol speelt bij de benutting en de stofwisseling van organische en anorganische bestanddelen in de beenderen, leek het waarschijnlijk dat vitamine-C bij de eischaalvorming een rol speelt in één van beide of beide fasen.

We kunnen dus veronderstellen, dat de werkelijke functie, die vitamine-C vervult, in wezen door de schildklier geregeld wordt.

Wat betreft de verkregen resultaten van de alkalireserve (volumeprocenten CO_2) in het plasma in de verschillende warme en koude klimaatsomstandigheden en met verschillende Na-toevoegingen, was het duidelijk dat de gebruikte organische Na-verbindingen de alkalireserve verhoogden. Dit betekent ook dat de dieren in een toestand van alkalosis verkeerden en dat het warme klimaat een lage alkalireserve vertoonde in vergelijking met een koud klimaat.

In geval van een gematigde temperatuur verhoogde de toevoeging van organische Na-bestanddelen ook de alkalireserve significant, wat gepaard ging met een significante verbetering in eigewicht en schaalkwaliteit. Deze verbetering bleek duidelijker, wanneer vitamine-C met natrium gecombineerd werd.

De enige veronderstelling ter verklaring van dit verschijnsel is, dat het bicarbonaatgehalte van het bloed is toegenomen, dat meer $CaCO_3$ is afgezet en dat het enzym carboxyanhydrase door alkalische stoffen in het bloed kan zijn geactiveerd.

Het blijkt dat nog veel meer onderzoek aangaande de problemen rond het zuur-base evenwicht bij leghennen verricht moet worden.

Aangaande de significante effecten op eigewicht en enkele schaalkenmerken bij gebruik van antibiotica bij gematigde temperaturen, kan mogelijk als verklaring dienen, dat antibiotica een remmende invloed op enkele darmbacteriën uitoefenen, hetgeen het stofwisselingsniveau en de calciumafzetting kan verbeteren.

Het is duidelijk dat leghennen bij hoge temperaturen eieren met een zwakke schaal produceren; dit werd aangetoond door een onderzoek van de microstructuur van de eischaal.

Het bleek dat eischalen van hennen, gehouden bij hoge omgevingstemperaturen een duidelijk verslechterde microstructuur in de verschillende lagen vertoonden (de cuticula, de spongieuze en de mamillaire laag en de schaalvliezen).

Er werden een onregelmatige kolomstructuur en tal van gaten waargenomen, die een zwakke spongieuze structuur veroorzaken. Dit was vanzelfsprekend het

gevolg van de verminderde calciumsecretie door de uterus bij hoge temperaturen.

De rol van vitamine-C bij de verbetering van enkele lagen, zoals de spongieuze laag, is een duidelijke weerspiegeling van een grotere calciumafzet in de schaal.

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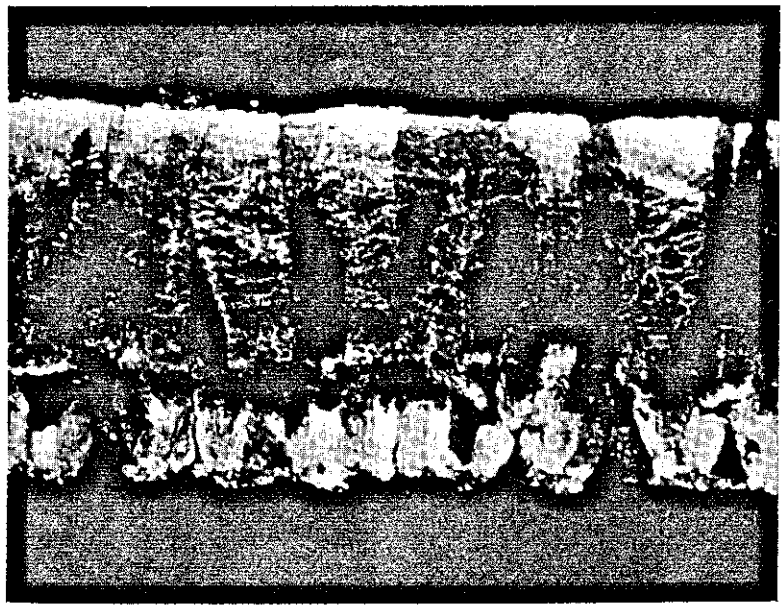

PLATE 1. Radial section plastic embedded showing a general view of a normal shell from a cold climate.
Note: the large, crystal columns, passing through the shell (Polarized light) × 125.

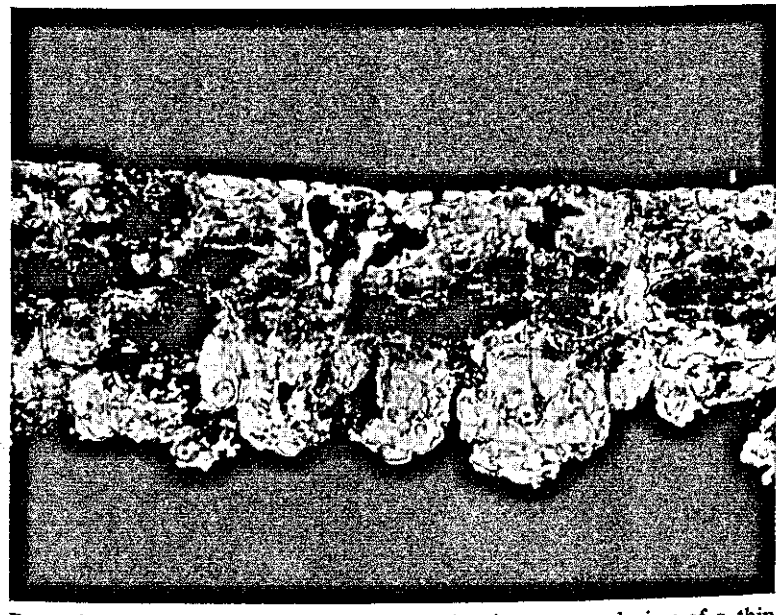

PLATE 2. Radial section plastic embedded showing a general view of a thin shell from an egg laid by a hot stressed hen.
Note: The deterioration in the different layers and the air gaps in the spongy layer (polarized light) × 125.

PLATE 3. Radial section plastic embedded showing a general view of a normal shell from a cold climate.
Note: the large, crystal columns, passing through the shell (polarized light) at a higher magnification × 300.

PLATE 4. Radial section plastic embedded showing a general view of a thin shell from an egg laid by a hot stressed hen.
Note: The deterioration in the different layers and the air gaps in the spongy layer (polarized light) at a higher magnification × 300.

PLATE 5. Radial plastic embedded section showing the spongy layer in a normal shell from a cold climate.
Note: The crystal columns running through the spongy layer (polarized light) × 300.

PLATE 6. Radial plastic embedded section showing the spongy layer of a thin shell from a hot stressed hen.
Note: The poor crystal structure of the spongy layer. Short irregular columns. Crystals are not running through the shell (polarized light) × 300.

PLATE 7. Radial plastic embedded section showing the mammillary knobs and shell membranes in a cold climate.
Note: The attachment of the mammillary knobs in the shell membranes by means of very fine protrusions (polarized light) × 125.

PLATE 8. Radial section plastic embedded giving a general view of a thin shell of an egg laid by a hot stressed hen.
Note: The deterioration of the different layers and the week attachments of the mammillary knobs in the shell membranes (polarized light) × 125.